PCT/US99/29743 Part 1 of 2

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : A61K 51/04, 51/08, 49/00, C07K 1/04 — A1

(11) International Publication Number: WO 99/10016

(43) International Publication Date: 4 March 1999 (04.03.99)

(21) International Application Number: PCT/CA98/00801

(22) International Filing Date: 21 August 1998 (21.08.98)

(30) Priority Data:

60/056,531	21 August 1997 (21.08.97)	US
2,214,704	5 September 1997 (05.09.97)	CA
60/067,403	5 December 1997 (05.12.97)	US

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(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published

With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: COMBINATORIAL LIBRARY

(57) Abstract

Provided herein are combinatorial libraries containing compounds of formula (1): A–(B)$_n$–C, wherein: A is a chelator complexed to metal or radionuclide metal; B is a spacer group; n is selected from the integers 0 and 1; and C comprises a mixture of potential targeting molecules. These libraries are useful in identifying labelled compounds which exhibit a desired targeting activity.

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therapy,[4-7] the use of Au complexes in rheumatoid arthritis therapy and the applications of Ga, In, Tc, Re, and Sm complexes in nuclear medicine.[3, 15-17]

Previously, difficulties have been encountered in attaching diagnostically useful metals and radionuclide metals to targeting agents. Targeting agents such as proteins and other macromolecules can offer the tissue specificity required for diagnostic accuracy. However, labeling of these agents with metal radionuclides is made difficult by their physical structure. Particularly, protein and peptide targeting agents present numerous sites at which radionuclide binding can occur, resulting in a product that is labeled heterogeneously. Also, and despite their possibly large size, proteins rarely present the structural configuration most appropriate for high affinity radionuclide binding, i.e. a region incorporating four or more donor atoms that form five-membered rings. As a result, radionuclides are bound typically at the more abundant low-affinity sites, forming unstable complexes.

We have found that a promising alternative to the direct labeling of targeting agents is an indirect approach, in which targeting agent and radionuclide are coupled using a chelating agent. Candidates for use as chelators are those compounds that bind tightly to the chosen metal radionuclide and also have a reactive functional group for conjugation with the targeting molecule.

However, it is difficult to identify suitable targeting agents that retain their targeting ability when coupled to a chelator complexed with a metal or a radionuclide metal. A major problem affecting radiopharmaceutical development arises from difficulties in incorporating diagnostically useful metal complexes such as ^{99m}technetium complexes into a targeting molecule without drastically reducing the affinity of the targeting molecule for the receptor and adequate pharmacokinetics. With targeting molecules that are small peptides or small organic compounds, the addition of a ch lated metal compound can double the molecular weight of the overall radiopharmaceutical and thereby radically alter the ability of the molecule to bind the receptor with comparable affinity.

To identify suitable labeled targeting agents according to the traditional approach, it is necessary to screen thousands of compounds in biological assays to provide a lead compound having the desired biological activity. This initial lead compound is then optimized to provide an agent having improved pharmacological properties. This approach is very time consuming and expensive.

The problems inherent in the traditional approach have been overcome for the development of some pharmaceuticals through the use of combinatorial chemistry. Combinatorial chemistry is a methodology by which large numbers of compounds or libraries can be prepared and screened rapidly and concurrently in an efficient manner.

While the use of combinatorial library techniques has been applied to the development of drugs that are organic molecules, it has never been applied to the development of metallodrugs. This is most likely due to the greater considerations involved in the chemistry of metallodrugs. In addition to the usual considerations, the oxidation state and the coordination chemistry of the metal, and the stability of the resulting metal complexes must also be considered when combinatorial library techniques are applied to metallodrug development. As the combinatorial library consists of a series of metal complexes, the site of metal coordination is of great importance. This site of metal coordination may be incorporated as part of the whole molecule or as a separate group or entity attached to the biologically active component of the molecule. In either case, the site of coordination affects the oxidation state of the metal, and vice versa. As the library of molecules will be evaluated in solution, the metal complexes must also resist decomplexation when they are cleaved from the solid support.

Attempts to overcome these problems have been made through the attachment of bifunctional chelators to moieties of potential biological activities. The use of bifunctional chelators permits control of the type of metal coordination, the oxidation state of the coordinated metal, the stability and the conformation of the

resulting metal compl x. A variety of bifunctional chelators are available. Examples of bifunctional chelators include polyamino polycarboxylates, polyamino polyphenolates, polyaza macrocycles with or without pendent coordination groups, tetradentate N_xS_{4-x} ligands, polyamino polyphosphates, polyamino polysulphides, polyamino polyheterocyclics and derivatives or combinations of the above mentioned chelators.[22-24] A number of techniques have been developed for attaching chelators to molecules of interest.[25-32]

However, problems in attaching stable metal complexes have not been adequately overcome because of the inability to produce chelators that provide for the stability necessary for the development of metallodrugs. As a result, the practice in this field has previously been to attach metal complexes to targeting molecules only after these molecules have been screened. As discussed above, the attachment of the metal complexes will often affect the binding of the lead targeting molecule to its receptor. This results in increased time and expense in searching for further lead molecules that do not lose their binding ability upon attachment to the chelated metal complex in question.

There is therefore a need for a method of producing a combinatorial library for isolating labeled radiopharmaceutical compounds that will bind to an appropriate receptor. There is a need for such a method that employs a suitable chelating agent that will permit the targeting molecule to be labeled prior to screening so that the labeled radiopharmaceutical compounds can be evaluated rapidly and efficiently to identify lead molecules. Such a method would significantly reduce the research and development effort required to identify new lead molecules.

Summary of the Invention

The present invention provides combinatorial library compounds which are ffective for binding to a biological target in a rapid and cost effective manner, as well as a method of synthesizing the compounds.

The present invention provides a combinatorial library of targeting agents that are labelled with a metal or radionuclide metal complexed to a chelating agent. A large number of labelled targeting agents can be quickly screened for their ability to bind to a biological target.

The present invention provides a combinatorial library of targeting compounds which have attached non-radioactive metal complexes which are isostructural with radiactive compounds for imaging applications or reactive Re complexes for radiotherapy.

According to one aspect of the present invention, there is provided a library comprising one or more sets of compounds, each set comprising a mixture of compounds of formula (1):

$A\text{-}(B)_n\text{-}C$ (1)

wherein: A is a chelator moiety capable of complexing a metal;
B is a spacer group;
n is selected from the integers 0 and 1; and
C comprises one of a plurality of potential targeting molecules.

According to another aspect of the present invention there is provided a library comprising one or more sets of compounds, each set comprising a mixture of compounds of formula (11):

$(W)_m\text{-}X\text{-}(Y)_n\text{-}Z$ (11)

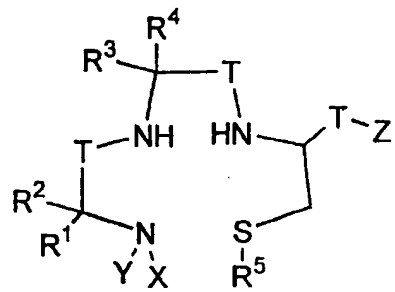
wherein: **W** is selected from a group comprising:

a) a metal binding moiety;

b) a chelator moiety capable of binding a metal selected from polyamino polycarboxylates, polyamino polyphenolates, polyazamacrocycles with or without pendent coordination groups, tetradentate N_xS_{4-x} ligands,

polyamino polysulfides, polyamino polyphosphates, polyamino polyheterocyclics and derivatives or combinations of the above;

c) a metal chelator of the general formula;



X is a linear or branched, saturated or unsaturated C_{1-6} alkyl chain that is optionally interrupted by one or two heteroatoms selected from N, O, and S; and is optionally substituted by at least one group selected from hydroxyl, amino, carboxyl, C_{1-6} alkyl, aryl and C(O)Z;

Y is H or a substituent defined by X;

Z is the position of attachment for the targeting portion of the library;

R^1 through R^4 are selected independently from H; carboxyl; C_{1-4} alkyl; C_{1-4} alkyl substituted with a group selected from hydroxyl, amino, sulfhydryl, halogen, carboxyl, C_{1-4} alkoxycarbonyl and aminocarbonyl; an alpha carbon side chain of a D- or L- amino acid other than proline; and C(O)Z;

R^5 is selected from H and a sulphur protecting group; and

T is carbonyl or CH_2.

d) a metal chelator selected from N,N-dimethyglycine-ser-cys-gly or N,N-dimethylglycine-tertbutylglycine-cys-gly; and

e) a chelator complexed to a metal or metal radionuclide;

X is a multiple chelator binding moiety capable of coupling to at least one metal binding moiety;

Y is a spacer group is selected from the integers 0 and 1;

Z comprises a mixture of potential targeting moieties;

m is greater than or equal to 1; and
n is selected from the integers 0 and 1.

According to another aspect of the present invention there is provided a method for the synthesis of a library comprising one or more sets of compounds, each set comprising a mixture of compounds of formula (1):

$A\text{-}(B)_n\text{-}C$ (1)

wherein: A is a chelator moiety capable of complexing a metal;
B is a spacer group;
n is selected from the integers 0 and 1; and
C comprises one of a plurality of potential targeting molecules.

comprising of the steps of:

(1) Preparing a mixture of potential targeting molecules using combinatorial synthesis;

(11) Attaching to the mixture a metal chelating moiety capable of complexing a metal; and

(111) Complexing the mixture with a solution of the metal in a suitable solvent.

According to another aspect of the present invention, there is provided a method for the synthesis of a library comprising one or more sets of compounds, each set comprising a mixture of compounds of formula (1):

$A\text{-}(B)_n\text{-}C$ (1)

wherein: A is a chelator moiety capable of complexing a metal;
B is a spacer group;
n is selected from the integers 0 and 1; and
C comprises one of a plurality of potential targeting molecules.

comprising the steps of:

(1) Preparing a mixture of potential targeting molecules using combinatorial synthesis; and

(11) Attaching to the mixture a preformed metal complex as an activated reagent in a suitable solvent.

According to yet another aspect of the present invention, there is provided a method of obtaining a compound having a desired targeting property comprising the steps of:

(1) providing a mixture which comprises a set of candidate compounds of formula (1):

$A\text{-}(B)_n\text{-}C$ (1)

wherein: A is a chelator complexed to a metal or metal nuclide
B is a spacer group
n is selected from the integers 0 and 1
C is one of a plurality of potential targeting molecules;

and

(11) selecting from amongst the set of candidate compounds a compound having the desired property by exposing the mixture of candidate compounds to a substance to which the compound having the desired targeting property will preferentially bind.

According to another aspect of the present invention there is provided a method of obtaining a labeled compound for the purposes of diagnostic imaging having a desired targeting property comprising the steps of:

(1) providing one or more sets of mixtures which comprise a mixture of candidate compounds of formula (1):

$A\text{-}(B)_n\text{-}C$ (1)

wherein: A is a chelator complexed to a metal or metal nuclide
B is a spacer group
n is selected from the integers 0 and 1

C is one of a plurality of potential targeting molecules;

and

(11) selecting from among the set of candidate compounds a compound having the desired property by exposing the mixture of candidate compounds to a substance to which the compound having the desired targeting property will preferentially bind.

According to another aspect of the present invention there is provided a method of obtaining a labeled compound for the purposes of therapy or radiotherapy having a desired targeting property comprising the steps of;

(1) providing one or more sets of mixtures which comprise a mixture of candidate compounds of formula (1):

$A\text{-}(B)_n\text{-}C$ (1)

Wherein: A is a chelator complexed to a metal or metal nuclide

B is a spacer group

n is selected from the integers 0 and 1; and

C is one of a plurality of potential targeting molecules;

and

(11) selecting from among the set of candidate compounds a compound having the desired property by exposing the mixture of candidate compounds to a substance to which the compound having the desired targeting property will preferentially bind.

According to yet another aspect of the present invention, there is provided a method of obtaining a compound having a desired targeting property comprising the steps of;

(1) providing a mixture or set of mixtures which comprise a set of candidate compounds of formula (11):

(W)m-X-(Y)n-Z (11)

wherein: W is a metal binding moiety

X is a multipl chelator binding moiety capable of coupling to at least one metal binding moiety

Y is a spacer group is selected from the integers 0 and 1; and

Z comprises a mixture of potential targeting moieties

m is greater than or equal to 1; and

n is selected from the integers 0 and 1; and

(11) selecting from among the set of compounds a compound having the desired targeting property by exposing the mixture of compounds to a substance to which the compound having a desired targeting property will preferentially bind.

According to another aspect of the present invention, there is provided a method of obtaining a molecule having a desired targeting property comprising the steps of:

(1) preparing a mixture or set of mixtures of candidate compounds of general formula (1):

$A\text{-}(B)_n\text{-}C$ (1)

Wherein: A is a chelator complexed to a non-radioactive metal which is isostructural with an analogous complex of a radioactive metal

B is a spacer group

n is selected from the integers 0 and 1; and

C is one of a plurality of potential targeting molecules;

(11) selecting from among the set of candidates a compound having the desired targeting property by exposing the mixture of candidate compounds to a substance to which the compound will preferentially bind; and

(111) preparing the isostructural radioactive analogue of the selected candidate having the desired targeting property

According to another aspect of the present invention, there is provided a method for the synthesis of a library comprising one or more sets of compounds, each set comprising a mixture of compounds of formula (1):

$A\text{-}(B)_n\text{-}C$ (1)

Wherein: A is a chelator complexed to a non-radioactive metal which is isostructural with an analogous complex of a radioactive metal;

B is a spacer group;

n is selected from the integers 0 and 1; and

C is one of a plurality of potential targeting molecules;

comprising the steps of:

(1) Preparing a mixture of potential targeting molecules using solid phase combinatorial synthesis; and

(11) Attaching to the mixture a preformed metal complex as an activated reagent in a suitable solvent.

According to another aspect of the present invention, there is provided a method for the synthesis of a library comprising one or more sets of compounds comprising the steps of:

(I) Selecting a suitable targeting molecule for binding a biological target;

(II) Preparing a library of non-radioactive rhenium-targeting molecule conjugates;

(III) Dividing mixtures of the conjugates into separate wells;

(IV) Assaying the mixtures for binding affinity to the biological target;

(V) Deconvoluting the mixtures having a high a binding affinity for said biological target; and

(VI) Isolating a series of discrete compounds having a high a binding affinity for said biological target.

Description

The invention provides an iterative approach of library synthesis followed by biological testing and subsequent deconvolution to provide final compounds. Following initial selection of a suitable target molecule, a moderately sized focused library of non-radioactive rhenium compounds is prepared as mixtures of up to 25 compounds. Typically, a large library of rhenium targeting moiety conjugates is delivered as equimolar mixtures of 9-25 compounds in 96 well microtiter plates (1mg/well) for *in vitro* testing. These are then tested in the relevant assays and the most promising mixtures are segregated for deconvolution. Depending on the number of promising molecules, discovered, a second round of testing may then be undertaken using a smaller subset of the rhenium containing molecules together with a second set of biological tests to further reduce the number of molecules. The final iteration will provide a series of discrete compounds as both the rhenium complex and a free chelate ready for labeling with radioactive ^{99m}technetium which is isostructural to the non-radioactive rhenium isotope used. The potential imaging lead candidates (preferably about 10 compounds) are delivered as pure chelator targeting moiety conjugates for radiolabeling development in *in vivo* studies. This process provides labeled compounds that are effective for binding a biological target in a rapid and cost effective manner.

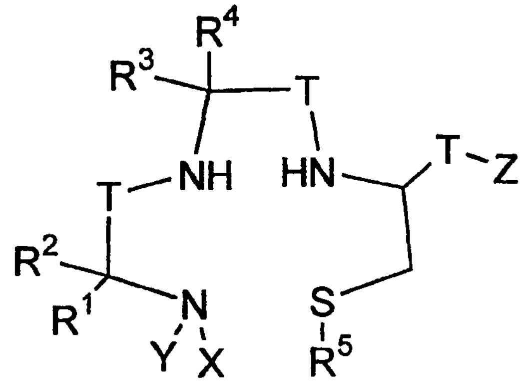
The targeting moiety of the present invention is a molecule that can selectively deliver a chelated metal or radionuclide or MRI contrasting agent to a desired location in a mammal. Preferred targeting molecules selectively target cellular receptors, transport systems, enzymes, glycoproteins and processes such as fluid pooling. Examples of targeting molecules suitable for coupling to the chelator include, but are not limited to: steroids, proteins, peptides, antibodies, nucleotides and saccharides. Preferred targeting molecules include proteins and peptides, particularly those capable of binding with specificity to cell surface receptors characteristic of a particular pathology. For instance, disease states associated with over-expression of particular protein receptors can be imaged by labeling that protein or a receptor

binding fragment th reof coupled to a suitable ch lator. Most preferably, targeting molecules are peptides capable of specifically binding to target sites and have three or more amino acid residues. The targeting moiety can be synthesized either on a solid support or in solution and is coupled to the next portion of the chelator-targeting moiety conjugates using known chemistry.

The second portion of the molecule, the optional linker, serves the purpose of separating the targeting portion from the imaging portion of the conjugate.

In the case of MRI agents, in order to increase the number of gadolinium (Gd) units attached to the biological target and for the purpose of increasing the relaxivity of the system, a multiple chelator coupling unit is attached to the targeting moiety (optionally via a linker subunit). This is of oligomeric or dendrimeric construction and is capable of coupling multiple chelator units to the conjugate. Preferably, this multiple chelator coupling unit is a dendrimer containing a functionality to which suitable chelators can be attached. Preferably, the multiple chelator coupling unit is a branched lysine dendrimer.

The metal chelators used for the purposes of the present invention have the following general formula:



wherein,

X is a linear or branched, saturated or unsaturated C_{1-6} alkyl chain that is optionally interrupted by one or two heteroatoms selected from N, O,and S; and is optionally substituted by at least one group selected from hydroxyl, amino, carboxyl, C_{1-6} alkyl, aryl and C(O)Z;

Y is H or a substituent defined by X;

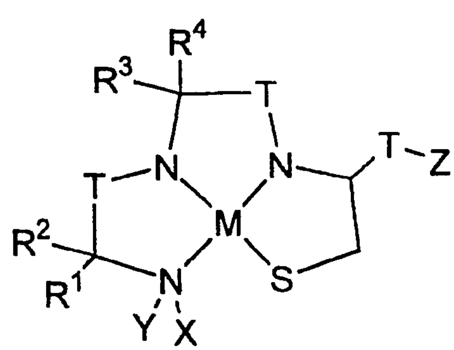
Z is the position of attachment for the targeting portion of the library;

R^1 through R^4 are selected independently from H; carboxyl; C_{1-4} alkyl; C_{1-4} alkyl substituted with a group selected from hydroxyl, amino, sulfhydryl, halogen, carboxyl, C_{1-4} alkoxycarbonyl and aminocarbonyl; an alpha carbon side chain of a D- or L- amino acid other than proline; and C(O)Z;

R^5 is selected from H and a sulphur protecting group; and

T is carbonyl or CH_2.

Where a chelator is complexed to a metal or a metal radionuclide, the complex has the following general formula:



X is a linear or branch d, saturated or unsaturated C_{1-8} alkyl chain that is optionally interrupted by one or two heteroatoms selected from N, O, and S; and is optionally substituted by at least one group selected from hydroxyl, amino, carboxyl, C_{1-8} alkyl, aryl and C(O)Z;

Y is H or a substituent defined by X;

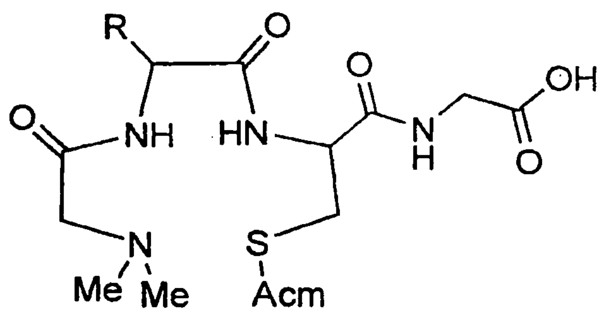
Z is the position of attachment for the targeting portion of the library;

R^1 through R^4 are selected independently from H; carboxyl; C_{1-4} alkyl; C_{1-4} alkyl substituted with a group selected from hydroxyl, amino, sulfhydryl, halogen, carboxyl, C_{1-4} alkoxycarbonyl and aminocarbonyl; an alpha carbon side chain of a D- or L- amino acid other than proline; and C(O)Z;

T is carbonyl or CH_2; and

M is metal

The preferred chelators for ^{99m}technetium radiopharmaceuticals are RP414 and RP455. The structures of RP414 and RP455 are as follows:



$R = CH_2OH$ RP414

$R = C(CH_3)_3$ RP455

Re and Tc complexes of these chelators are isostructural. Also, these chelators are advantageous because the chemistry of these compounds is well understood and they form neutral Re and ^{99m}Tc complexes. It is possible to label these chelators with Re or ^{99m}Tc in one easy step. In addition these chelators have

the advantage of being applicable for conjugation to a variety of targeting molecules, being compatible with solid phase synthesis.

Labeling of RP414 with ^{99m}Tc can be carried out either at ambient or elevated temperature, rapidly, and with quantities of chelator approaching stoichiometric amounts. The complex is stable to both acidic and basic conditions and remains unchanged *in-vivo.*

Other chelators may be used to carry out the invention. The invention is not limited to the preferred chelators listed above.

With MRI agents, the chelator comprises a functionality chosen from the known Gd chelators and is attached to the remainder of the conjugate by either solid phase or solution chemistry.

The following examples illustrate further this invention. Abbreviations used in the examples include Acm: acetoamidomethyl; Arg: arginine; Boc: *tert*-butyloxycarbonyl; Cys: cysteine; DIEA: diisopropylethylamine; Dimethylgly: N,N-dimethylglycine; DMF: N,N-dimethylformamide; ES-MS: Electron Spray Mass Spectrometry; Fmoc: 9-fluorenylmethyloxycarbonyl; Gly: glycine; HBTU: 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyl-uronium hexafluorophosphate; HOBT: 1-hydroxybenzotriazole; HPLC: high performance liquid chromatography; Leu: leucine; Lys: lysine; mL: millilitre(s); mmol: millimole(s); mol: mole(s); Mott: 4-methoxytrityl; NMP: N-methylpyrrolidone; Phe: phenylalanine; Pmc: 2,2,5,7,8-pentamethylchroman-6-sulfonyl; R_t: retention time; sasrin: 2-methoxy-4-alkoxybenzyl alcohol (super acid sensitive resin); Ser: serine; *t*-Bu: *tert*-butyl; TFA: trifluoroacetic acid; Thr: threonine; Trt: trityl; Tyr: tyrosine; Y^e-R: protection group R is attached to the peptide chain via the atom, Y, on the amino acid side chain (Y is N, O or S and R is Acm, Boc, Mott, *t*-Bu or Trt). N-methylpyrrolidone, dimethylformamide, 2-(1H-benzotriazol-1-yl)-1,1,3,3-tetramethyl-uronium hexafluorophosphate, 1-hydroxybenzotriazole, diisopropylethyl-amine, dichloromethane and trifluoroacetic

acid were purchased from Applied Biosystems Inc. Triethylamine and *tert*-butyl methyl ether were purchased from Aldrich Ch mical Inc. Fmoc amino acids and Sasrin resin were purchased from Bachem Bioscience Inc. All chemicals were used as received. $[ReO_2(en)_2]Cl$ and $ReOCl_3(PPh_3)_2$ were prepared according to literature methods (Rouschias, G. *Chem. Rev.* **1974**, *74*, 531; Fergusson, J. E. *Coord. Chem. Rev.* **1966**, *1*, 459). Mass spectra (electrospray) were obtained on a Sciex API#3 mass spectrometer in the positive ion detection mode. HPLC analyses and purifications were made on a Beckman System Nouveau Gold chromatographic system with a Waters 4 mm radial pak C-18 column. During analytical HPLC analysis, the mobile phase was changed from 100% 0.1% aqueous trifluoroacetic acid to 100% acetonitrile containing 0.1% trifluoroacetic acid over 20 minutes at a flow rate of 2 mL/min. The HPLC analyses of the RP487 peptide mixture and the Re complexes of the peptide mixture were performed by changing the mobile phase from 100% 0.1% aqueous trifluoroacetic acid to 60% acetonitrile containing 0.1% trifluoroacetic acid over 20 minutes at a flow rate of 2 mL/min. All HPLC analyses were monitored with a UV detector set at 215 and 254 nm. Solid phase peptide syntheses were performed on an ABI Peptide Synthesizer model 433A using FastMoc chemistry and sasrin resin (*User' Manual of Peptide Synthesizer Model 433A*, Applied BioSystems, Philadelphia, **1993**).

Example 1: **Preparation of a Re or Tc receptor specific radiopharmaceutical**

In preparing a combinatorial library of Re or ^{99m}Tc receptor specific radiopharmaceuticals, a set of potentially receptor specific structurally distinct molecules are placed on a solid support. A bifunctional chelator such as dimethylglycine-serine-cysteine-glycine is attached to the library of molecules using tetrafluorophenol and 1-[3-(dimethylamino)-propyl]3-ethylcarbodiimide chloride. The resulting solid phase library is then heated in a solution of $ReOCl_3(PPh_3)_2$ or $[ReO_2(en)_2]Cl$ to produce a library of Re complexes. The library of ^{99m}Tc complexes can be prepared by reacting the library with pertechnetate in the presence of tin (II) chloride and sodium gluconate. Alternatively, the library of Re and ^{99m}Tc complexes

can be prepared by reacting the set of potentially receptor specific molecules with the tetrafluorophenol esters of Re and Tc dimethglycine-serine-cysteine-glycine complexes. The libraries of Re and ^{99m}Tc complexes are then cleaved off the solid support and evaluated in biological assay or in imaging studies.

Example 2: **Synthesis of Gd complex as a receptor specific MRI contrasting agent.**

Upon deciding on a particular receptor, a series of potentially receptor specific structurally distinct molecules are attached to solid phase support. Using diethylenetriaminetetraacetic acids dianhydride, the chelator diethylenetriaminetetraacetic acid (DTPA) is attached to the set of potentially receptor specific molecules. The resulting solid phase library is then placed in a solution of Gd acetate to produce a solid phase library of potentially receptor specific Gd complexes. The Gd is coordinated to the DTPA chelator. The library of Gd complexes is then cleaved off the solid support and evaluated in a biological assay.

Example 3: **Development of a magnetic resonance imaging agent.**

Some current attempts to produce efficient relaxation have resulted in the preparation of molecules having a number of gadolinium chelator molecules attached to one targeting molecule, often by way of a linker moiety to allow space between the target and the chelation parts of the molecule[37-39].

The molecule can be divided into four parts; the targeting moiety, an optional suitable linker, a multiple chelator coupling unit capable of coupling multiple chelator moieties, and the chelator moieties coupled to the multiple chelator coupling unit.

The targeting moiety is a molecule that can selectively deliver a chelated radionuclide or MRI contrasting agent to a desired location in a mammal.

The second portion of the molecule, the optional linker, serves the purpose of separating the targeting portion from th imaging portion of th conjugate.

In order to increase the number of gadolinium units attached to the biological target and for the purpose of increasing the relaxivity of the system a multiple chelator coupling unit is attached to the targeting moiety (optionally via a linker subunit). This is of oligomeric or dendrimeric construction and is capable of coupling multiple chelator units to the conjugate. Preferably, this multiple chelator coupling unit is a dendrimer containing a functionality to which suitable chelators can be attached. Preferably, the multiple chelator coupling unit is a branched lysine dendrimer.

The final portion of the conjugate will consist of the chelator units. This comprises a functionailty chosen from the known Gd chelators and is attached to the remainder of the conjugate by either solid phase or solution chemistry.

In order to synthesize the combinatorial libraries, mixtures of compounds in any or all of the above subsections of the agent are prepared. In addition, the preparation of a combination of libraries having a mixture in one of the sections, together with a series of related libraries produced by the alteration of the previous or subsequent parts of the agent is carried out in a parallel fashion. Hence, a library of targeting molecules would be split and each part attached to a single linker-dendrimer-chelator subunit. Such a route provides a parallel series of libraries each having a single linker-dendrimer-chelator unit in order to optimize the targeting of the molecules. Producing a mixture of compounds based on the dendrimer and single targeting-linker and chelator units allows for variation of the relaxivity of the system.

Example 4: **Synthesis of Peptides Attached to a Solid Polymer Resin.**

Peptides of various amino acid sequences and with varying side chain protection groups were prepared via a solid phase peptide synthesis method on an

automated peptide synthesizer using FastMoc 1.0 mmole chemistry.[3] Preloaded Fmoc amino acid sasrin resin and Fmoc amino acid derivatives w re used. Prior to the addition of each amino acid residue to the N-terminus of the peptide chain, the FMOC group was removed with 20% piperidine in NMP. Each Fmoc amino acid residue was activated with 0.50 M HBTU/ HOBt/ DMF, in the presence of 2.0M DIEA/ NMP. The C-terminus of the completed peptide was attached to the resin via the sasrin linker. The peptidyl resin was washed with dichloromethane and dried under vacuum for 20-24 hours. This method was used to prepare the following peptidyl resin of varying amino acid sequences containing side chain protection groups:

1)RP414-resin:	Dimethylgly-Ser(O^e-*t*-Bu)-Cys(S^e-Acm)-Gly-[resin]
2)RP440-resin:	Dimethylgly-Ser(O^e-*t*-Bu)-Cys(S^e-Trt)-Gly-[resin]
3)RP441-resin:	Dimethylgly-Ser(O^e-Trt)-Cys(S^e-Mott)-Gly-Thr-Lys(N^e-Boc)-Pro-Pro-Arg(N^e-Pmc)-[resin]
4)RP442-resin:	Dimethylgly-Ser(O^e-Trt)-Cys (S^e-Trt)-Gly-[resin]
5)RP443-resin:	Dimethylgly-Ser(O^e-Trt)-Cys(S^e-Mott)-Gly-[resin]
6)RP478-resin:	Dimethylgly-Ser(O^e-Trt)-Cys(S^e-Mott)-Gly-Gly-Lys(N^e-Boc)-Lys(N^e-Boc)-Leu-Leu-Lys(N^e-Boc)-Lys(N^e-Boc)-Leu-Lys(N^e-Boc)-Lys(N^e-Boc)-Leu-Leu-Lys(N^e-Boc)-Lys(N^e-Boc)-Leu-NH_2-[resin]

<u>Example 5:</u> **Synthesis of RP487 peptide mixture-resin, Dimethylgly-Ser(O^e-Trt)-Cys(S^e-Mott)-Gly-*X*-Tyr(O^e-*t*-bu)-Gly-*Z*-Gly-[resin] (where *X* are Leu, Arg(N^e-Pmc) or Phe and *Z* are Lys(N^e-Boc), Ser(O^e-Trt) or Tyr(O^e-*t*-bu)).**

The synthesis of the RP487 peptide mixture resin was performed using FastMoc 0.25 mmol chemistry on an automated synthesizer.[35] Fmoc-Gly sasrin-resin (0.7 mmol/ g, 0.25 mmol, 357 mg) was placed in the reaction vessel. Amino acid cartridges 2, 3, 5, 6, and 7 counted from the C-terminus contained 1 mmol of each Fmoc amino acid derivatives, Gly, Tyr(O^e-t-Bu), Gly, Cys(S^e-Mott), and

Ser(O^e-Trt), respectively. Cartridge 1 had Fmoc amino acid derivatives of Lys(N^e-Boc), Ser(O^e-Trt), and Tyr(O^e-t-Bu) (0.33 mmol / amino acid). Meanwhile, cartridge 4 carried Fmoc amino acids of Arg(N^e-Pmc), Leu, and Phe (0.33 mmol/ amino acid). N,N-Dimethylglycine (1mmol) in cartridge 8 was pre-treated with 0.50 M HBTU/ HOBt/ DMF (0.8 mL) before it was inserted on the synthesizer. After completion of the automatic synthesis, the resulting product was removed from the synthesizer, and dried under vacuum for 2 hours to afford the titled RP487 peptide mixture-resin (610 mg).

Example 6: **Synthesis of RP487 peptide mixture, Dimethylgly-Ser-Cys-Gly-*X*-Tyr-Gly-*Z*-Gly (where *X* is Leu, Arg or Phe and *Z* is Lys, Ser or Tyr).**

The RP487 peptide mixture-resin (120 mg, 0.05 mmol) was added to a cleavage composition of 82.5% TFA/ 5% phenol/ 5% thioanisole/ 2.5% 1,2-ethane dithiol/ 5% mili-Q water (1 mL) at 0 °C.[36] The reaction suspension was then stirred at room temperature for 5 hours. The cleavage suspension was filtered by vacuum after 5 hours, and the filtrate was allowed to add in cold *tert*-butyl methyl ether (20 mL) at 5°C. The precipitated residue was subsequently washed with *tert*-butyl methyl ether (2 x 30 mL). The resulting residue was then dissolved in milli-Q water (2 mL), frozen and lyophilized (22 hours) to give the titled RP487 peptide mixture as an off-white pellet (52 mg). Mass spectrum (electrospray): m/z = 828 (MH^+, [$C_{34}H_{54}N_9O_{13}S_1$]), {X is Leu and Z is Ser}; m/z = 862 (MH^+, [$C_{37}H_{52}N_9O_{13}S_1$]), {X is Phe and Z is Ser};. m/z = 869 (MH^+, [$C_{37}H_{61}N_{10}O_{12}S_1$]), {X is Leu and Z is Lys}; m/z = 871 (MH^+, [$C_{34}H_{55}N_{12}O_{13}S_1$]), {X is Arg and Z is Ser}; m/z = 903 (MH^+, [$C_{40}H_{59}N_{10}O_{12}S_1$]), {X is Phe and Z is Lys}; m/z = 904 (MH^+, [$C_{40}H_{58}N_9O_{13}S_1$]), {X is Leu and Z is Tyr}; m/z = 912 (MH^+, [$C_{37}H_{62}N_{13}O_{12}S_1$]), {X is Arg and Z is Lys }; m/z = 938 (MH^+, [$C_{43}H_{56}N_9O_{13}S_1$]), {X is Phe and Z is Tyr }; m/z = 947 (MH^+, [$C_{40}H_{59}N_{12}O_{13}S_1$]), {X is Arg and Z is Tyr }. HPLC retention time: R_t = 7.18 minutes (broad peak); R_t = 9.0-9.9 minutes (overlapping broad peak); R_t = 11.0-11.5 minutes (overlapping broad peak); R_t = 16.5-16.8 minutes (broad overlapping peaks); R_t = 17.9 minutes (broad peaks).

Example 7: **Synth sis of Re oxo complex of Dimethylgly-S r-Cys-Gly using RP442-resin.**

RP442-resin (0.1055 g) was swollen in 3 mL of NMP. $ReOCl_3(PPh_3)_2$ (0.3793 g, 0.000456 moles) and 1 mL of triethylamine were added to the NMP resin mixture. The $ReOCl_3(PPh_3)_2$ dissolved to give a purple mixture. The mixture was heated at 40-50 °C for 4 hours. The resin was then collected by vacuum filtration. The resin was washed with 3 x 10 mL NMP, followed by 3 x 10 mL CH_2Cl_2. The resin was dried under vacuum overnight. To cleave the Re complex off the solid support, the resin was placed in 90 % aqueous trifluoroacetic acid and stirred for 4 hours. The resin was removed by vacuum filtration and the supernatant was added dropwise to *tert*-butyl methyl ether at 0 °C. Red-brown precipitate formed. The precipitate was collected by centrifugation and analyzed by HPLC and electrospray mass spectrometry. The *syn* and *anti* isomers of the Re complex were observed in the HPLC chromatogram. This is consistent with other known Re complexes with $N_{4-x}S_x$ chelators. The coordination of the peptide to the Re metal caused the displacement of the cysteine sulfur trityl protection group but did not cause the peptide to be cleaved off the resin. The cleavage of the peptidic complex off the resin in acidic medium removed all remaining side chain protection groups but did not cause the metal complex to undergo decomplexation.. Mass spectrum (electrospray): m/z = 550 (MH^+, $[C_{12}H_{20}N_4O_7Re_1S_1]$). HPLC retention time: R_t = 5.98 minutes (isomer A); R_t = 6.22 minutes (isomer B).

Example 8: **Synthesis of Re oxo complex of Dimethylgly-Ser-Cys-Gly using RP443-resin.**

RP443-resin (0.1621 g) was swollen in 3 mL of NMP. $ReOCl_3(PPh_3)_2$ (0.4023 g, 0.000483 moles) and 1 mL of triethylamine were added to the NMP resin mixture. The $ReOCl_3(PPh_3)_2$ dissolved to give a purple mixture. The mixture was heated at 40-50 °C for 4 hours. The resin was then collected by vacuum filtration. The resin

was washed with 3 x 10 mL NMP, followed by 3 x 10 mL CH_2Cl_2. The resin was dried under vacuum overnight. To cleave the Re complex off the solid support, the resin was placed in 90 % aqueous trifluoroacetic acid and stirred for 4 hours. The resin was removed by vacuum filtration and the supernatant was added dropwise to *tert*-butyl methyl ether at 0 °C. Red-brown precipitate formed. The precipitate was collected by centrifugation and analyzed by HPLC and electrospray mass spectrometry. The *syn* and *anti* isomers of the Re complex was observed in the HPLC chromatogram. The coordination of the peptide to the Re metal caused the displacement of the cysteine sulfur trityl protection group but did not cause the peptide to be cleaved off the resin. The cleavage of the peptidic complex off the resin in acidic medium removed all remaining side chain protection groups but did not cause the metal complex to undergo decomplexation. Mass spectrum (electrospray): m/z = 550 (MH^+, $[C_{12}H_{20}N_4O_7Re_1S_1]$). HPLC retention time: R_t = 5.53 minutes (isomer A); R_t = 6.13 minutes (isomer B).

Example 9: **Synthesis of Re oxo complex of Dimethylgly-Ser-Cys-Gly-Thr-Lys-Pro-Pro-Arg using RP441-resin.**

RP441-resin (0.0799 g) was swollen in 3 mL of NMP. $ReOCl_3(PPh_3)_2$ (0.3983 g, 0.000478 moles) and 1 mL of triethylamine were added to the NMP resin mixture. The $ReOCl_3(PPh_3)_2$ dissolved to give a purple mixture. The mixture was heated at 40-50 °C for 8 hours. The resin was then collected by vacuum filtration. The resin was washed with 3 x 10 mL NMP, followed by 3 x 10 mL CH_2Cl_2. The resin was dried under vacuum overnight. To cleave the Re complexes off the solid support, the resin was placed in 90 % aqueous trifluoroacetic acid and stirred for 4 hours. The resin was removed by vacuum filtration and the supernatant was added dropwise to *tert*-butyl methyl ether at 0 °C. Red-brown precipitate formed. The precipitate was collected by centrifugation and analyzed by HPLC and electrospray mass spectrometry. The *syn* and *anti* isomers of the Re complex were observed in the HPLC chromatogram. The coordination of the peptide to the Re metal caused the displacement of the cysteine sulfur trityl protection group but did not cause the

peptide to be cleaved off the resin. The cleavage of the peptidic complex off the resin in acidic medium removed all remaining side chain protection groups but did not cause the metal complex to undergo decomplexation. Mass spectrum (electrospray): m/z = 1129 (MH^+, [$C_{38}H_{65}N_{13}O_{13}Re_1S_1$]). HPLC retention time: R_t = 6.18 minutes (isomer A); R_t = 6.70 minutes (isomer B).

Example 10: **TKPPR+ReORP455-TP-P ester:**

To a slurry of sasrin-Arg-Pro-Pro-Lys-Thr resin (15mg) in ethyl acetate (0.5mL) was added a solution of ReO-N,Ndimethylglycine-ser-cys-gly-Otfp (prepared from 10mg of ReO-N,N-dimethyglycine-ser-cys-gly-OH) in ethyl acetate (0.5mL) and the resulting solution shaken intermittently at room temperature for 2h. The resulting colourless solution was filtered off from the now pink/brown resin and the resin washed with ethyl acetate followed by dichloromethane and then dried in vacuo. The peptide-chelate conjugate was then liberated from the resin by treatment with 0.5mL 95% trifluoroacetic acid at room temperature for 1.5h. The solution was then filtered off and the volatiles removed under reduced pressure. Presence of ReORP128 was confirmed by co-injection of the product with an authentic sample of ReORP128 prepared by solution chemistry (retention time 9.15 using a gradient elution of 0 to 100% acetonitrile in water buffered with 0.1% trifluoroacetic acid over 20 mins)

Example 11: **Synthesis of Re oxo complex of Dimethylgly-Ser-Cys-Gly-Gly-Lys-Lys-Leu-Leu-Lys-Lys-Leu-Lys-Lys-Leu-Leu-Lys-Lys-Leu-NH_2 using RP478-resin.**

RP478-resin (0.1125 g) was swollen in 3 mL of NMP. $ReOCl_3(PPh_3)_2$ (0.6351 g, 0.000763 moles) and 1 mL of triethylamine were added to the NMP resin mixture. The $ReOCl_3(PPh_3)_2$ dissolved to give a purple mixture. The mixture was heated at 40-50 °C for 8 hours. The resin was then collected by vacuum filtration. The resin was washed with 3 x 10 mL NMP, followed by 3 x 10 mL CH_2Cl_2. The resin was

dried under vacuum overnight. To cleave the Re complex off the solid support, the resin was placed in 90 % aqueous trifluoroacetic acid and stirred for 8 hours. The resin was removed by vacuum filtration and the supernatant was added dropwise to *tert*-butyl methyl ether at 0 °C. Red-brown precipitate formed. The precipitate was collected by centrifugation and analyzed by HPLC and electrospray mass spectrometry. The *syn* and *anti* isomers of the Re complex was observed in the HPLC chromatogram. The coordination of the peptide to the Re metal caused the displacement of the cysteine sulfur trityl protection group but did not cause the peptide to be cleaved off the resin. The cleavage of the peptidic complex off the resin in acidic medium removed all remaining side chain protection groups but did not cause the metal complex to undergo decomplexation. Mass spectrum (electrospray): m/z = 2310 (MH^+, [$C_{98}H_{188}N_{28}O_{21}Re_1S_1$]). HPLC retention time: R_t = 11.17 minutes (broad peak).

Example 12: **Synthesis of Re oxo complex of Dimethylgly-Ser-Cys-Gly-*X*-Tyr-Gly-*Z*-Gly (where *X* is Leu, Arg or Phe and *Z* is Lys, Ser or Tyr) using RP487-resin.**

RP487-resin (0.0618 g) was swollen in 3 mL of NMP. $ReOCl_3(PPh_3)_2$ (0.9210 g, 0.00111 moles) and 1 mL of triethylamine were added to the NMP resin mixture. The $ReOCl_3(PPh_3)_2$ dissolved to give a purple mixture. The mixture was heated at 40-50 °C for 12 hours. The resin was then collected by vacuum filtration. The resin was washed with 3 x 10 mL NMP, followed by 3 x 10 mL CH_2Cl_2. The resin was dried under vacuum overnight. To cleave the Re complexes off the solid support, the resin was placed in 90 % aqueous trifluoroacetic acid and stirred for 4 hours. The resin was removed by vacuum filtration and the supernatant was added dropwise to *tert*-butyl methyl ether at 0 °C. Red-brown precipitate formed. The precipitate was collected by centrifugation and analyzed by HPLC and electrospray mass spectrometry. Since the Re complex of each peptide sequence exists as the *syn* and *anti* isomers, the total number of compounds prepared was 18. Mass spectrum (electrospray): m/z = 1027 (MH^+, [$C_{34}H_{51}N_9O_{14}Re_1S_1$]), {X is Leu and Z is

Ser}; m/z = 1062 (MH^+, [$C_{37}H_{50}N_9O_{14}Re_1S_1$]), {X is Phe and Z is Ser};. m/z = 1069 (MH^+, [$C_{37}H_{59}N_{10}O_{13}Re_1S_1$]), {X is Leu and Z is Lys}; m/z = 1071 (MH^+, [$C_{34}H_{53}N_{12}O_{14}Re_1S_1$]), {X is Arg and Z is Ser}; m/z = 1103 (MH^+, [$C_{40}H_{57}N_{10}O_{13}Re_1S_1$]), {X is Phe and Z is Lys}; m/z = 1104 (MH^+, [$C_{40}H_{56}N_9O_{14}Re_1S_1$]), {X is Leu and Z is Tyr}; m/z = 1112 (MH^+, [$C_{37}H_{60}N_{13}O_{13}Re_1S_1$]), {X is Arg and Z is Lys }; m/z = 1138 (MH^+, [$C_{43}H_{54}N_9O_{14}Re_1S_1$]), {X is Phe and Z is Tyr }; m/z = 1147 (MH^+, [$C_{40}H_{67}N_{12}O_{14}Re_1S_1$]), {X is Arg and Z is Tyr }. HPLC retention time: R_t = 8.40 minutes; R_t = 8.99 minutes; R_t = 9.62 minutes; R_t = 9.90 minutes; R_t = 10.14 minutes; R_t = 11.0-12.7 minutes (overlapping peaks); R_t = 15.1 minutes (broad peak); R_t = 15.4 minutes (broad peak).

<u>Example 13:</u> **Synthesis of Re oxo complex of Dimethylgly-Ser-Cys-Gly-*X*-Tyr-Gly-*Z*-Gly (where *X* are Leu, Arg or Phe and *Z* are Lys, Ser or Tyr) in aqueous solution.**

[$ReO_2(en)_2$]Cl (0.0434 g, 0.000116 moles) was dissolved in 1.5 mL of milli-Q water. The mixture of the 9 peptides (0.0436 g) was dissolved in 2 mL of milli-Q water. The two solutions were combined to give a light green solution. The pH of the solution was adjusted to 6 using 1 M NaOH. The solution was refluxed under Ar for 2 hours, during which time the solution changed from green to red. The solution was frozen and lyophilized overnight, yielding a red solid. The solid was analyzed by HPLC and electrospray mass spectrometry. Mass spectrum (electrospray): m/z = 1027 (MH^+, [$C_{34}H_{51}N_9O_{14}Re_1S_1$]), {X is Leu and Z is Ser}; m/z = 1061 (MH^+, [$C_{37}H_{49}N_9O_{14}Re_1S_1$]), {X is Phe and Z is Ser};. m/z = 1068 (MH^+, [$C_{37}H_{58}N_{10}O_{13}Re_1S_1$]), {X is Leu and Z is Lys}; m/z = 1071 (MH^+, [$C_{34}H_{53}N_{12}O_{14}Re_1S_1$]), {X is Arg and Z is Ser}; m/z = 1103 (MH^+, [$C_{40}H_{57}N_{10}O_{13}Re_1S_1$]), {X is Phe and Z is Lys}; m/z = 1104 (MH^+, [$C_{40}H_{56}N_9O_{14}Re_1S_1$]), {X is Leu and Z is Tyr}; m/z = 1112 (MH^+, [$C_{37}H_{60}N_{13}O_{13}Re_1S_1$]), {X is Arg and Z is Lys }; m/z = 1138 (MH^+, [$C_{43}H_{53}N_9O_{14}Re_1S_1$]), {X is Phe and Z is Tyr }; m/z = 1146 (MH^+, [$C_{40}H_{56}N_{12}O_{14}Re_1S_1$]), {X is Arg and Z is Tyr }. HPLC retention time: R_t = 8.45 minutes; R_t = 9.02 minutes; R_t = 9.67 minutes;

R_t = 9.95 minutes; R_t = 10.27 minutes; R_t = 11.0-12.8 minutes (overlapping peaks); R_t = 15.2 minutes (broad peak); R_t = 15.7 minutes (broad peak).

Example 14: **Use of multiple discrete loaded resins in one reactor vessel to provide a combinatorial library**

The various peptide sequences containing varying side chain protecting groups in these examples were synthesized via a solid phase synthesis method on an automated synthesizer using FastMoc chemistry on scales varying from 0.1 to 1.0mmol. Prior to the addition of each amino acid residue to the N-terminus of the peptide chain the FMOC group was removed with 20% piperidine in NMP. Each FMOC amino acid was activated with 0.5M HOBT/HBTU/DMF in the presence of 2.0M DIEA/NMP. The C-terminus was attached to the solid phase *via* the sasrin linker. After completion of the synthesis the resin was washed with NMP followed by dichloromethane and dried under vacuum for up to 24 hours.

Example 15: **Synthesis of a library of peptides on solid phase having the sequence Dimethylglycine-Ser(O-Trt)-Cys(S-Acm)-Gly-X-Tyr(O-t-Bu)-Gly-Z-Y (Where X is Leu, Arg(N-Pmc), or Phe, Z is Lys(N-Boc), Ser(O-Trt), or Tyr(o_t-Bu), and Y is Gly, Phe, Leu, Arg(N-Pmc), or Lys(N-Boc))**

The reactor vessels employed in the peptide synthesizer were loaded with a mixture of 5 MicroKans (supplied by IRORI) each containing 30mg of Tenta gel TGA resin having the following amino acids preloaded; glycine, Phenylalanine, Leucine, Arginine, Lysine. This mixture was then subjected to the conditions described above to synthesize the following amino acid sequence onto each of the resins;

RPLIB6G-resin: Dimethylgly-ser(O-t-bu)-Cys(S-Acm)-Gly-X-Tyr(O-t-Bu)-Gly-Z-Gly-Resin

RPLIB6F-resin	Dimethylgly-ser(O-t-bu)-Cys(S-Acm)-Gly-X-Tyr(O-t-Bu)-Gly-Z-Phe-Resin
RPLIB6L-resin:	Dimethylgly-ser(O-t-bu)-Cys(S-Acm)-Gly-X-Tyr(O-t-Bu)-Gly-Z-Leu-Resin
RPLIB6R-resin:	Dimethylgly-ser(O-t-bu)-Cys(S-Acm)-Gly-X-Tyr(O-t-Bu)-Gly-Z-Arg-Resin
RPLIB6K-resin:	Dimethylgly-ser(O-t-bu)-Cys(S-Acm)-Gly-X-Tyr(O-t-Bu)-Gly-Z-Lys-Resin

Where X consists of a mixture of FMOC amino acid derivatives of Leu, Arg (N-Pmc), or Phe, and Z consists of a mixture of FMOC amino acid derivatives of Lys(N-boc), Ser(O-trt) or Tyr(O-t-Bu). These mixture are incorporated into the peptide synthesis using the method outlined in example 2

Example 16: **Synthesis of a series of peptides having the sequence Dimethylglycine-Ser-Cys-Gly-X-Tyr-Gly-Z-Y (Where X is Leu, Arg, or Phe, Z is Lys, Ser, or Tyr, and Y is Gly, Phe, Leu, Arg, or Lys)**

The RPLIB6 mixtures prepared in example 15 above (30mg) were added to a cleavage mixture consisting of 95% TFA in water (300 L). The reaction suspensions were then shaken at room temperature for 3hours. The mixtures were then filtered and added to tert-butyl methyl ether (1mL). The resulting solid was collected and dried under vacuum then analysed by HPLC using a method of 0 to 100% acetonitrile in water buffered with 0.1% trifluoroacetic acid. Comparison of the particular peptide sequence where Y is Gly with that peptide synthesized in example 6 by HPLC showed formation of the required compounds.

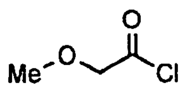
Example 17: **Investigation f the eff ct f amid capping gr up (CG)NH on the agonist/antagonist functioning ability f p ptid CG-NH-Nle-Leu-Leu-Phe-Lys-Gly-COOH**

The N-terminal amine of the above peptide attached to sasrin resin (100mg) is deprotected of its Fmoc group under standard Fmoc deprotection conditions. The amine is then capped with a suitably reactive reagent (see list below). The resin is placed in a solution of NMP (500uL) for the resin to swell. To this solution is added DIEA (25uL, 2M) and one of the capping agents (0.5mmol). The reaction is shaken for 2 to 18 hours at room temperature. The reaction is filtered and the resin is washed with NMP (3x5mL) then dichloromethane (3x5mL) and the resin is dried in vacuo. The capped peptide is removed from the resin by shaking in the presence of 95% Trifluoroacetic acid (0.5-1mL) at room temperature for 4 hours. After the cleavage is complete the resin is filtered off and then washed with further trifluoroacetic acid (0.5mL). The combined trifluoroacetic acid solutions are then combined and concentrated under reduced pressure. The capped peptide is then purified by reverse phase HPLC on C18 silica gel using a gradient of 0-100% acetonitrile in water over a period of 20 mins. The cuts containing the relevant compound are then lyophilized and the peptide analyzed by electrospray mass spectroscopy. The peptides display the properties shown in the table below.

RP#	Capping Group #	Retention Time	Molecular Weight
522-2	2	11.31	592
522-3	3	12.07	614
522-4	4	13.24	670
522-6	6	12.69	619
522-7	7	12.92	618
522-10	10	13.06	653
522-12	12	11.76	738

522-13	13	14.50	682
522-14	14	12.89	676
522-15	15	13.23	650
522-16	16	14.96	742
522-17	17	11.70	588
522-18	18	14.13	715

A list of the amine capping agents (and the associated capping group number) includes but is not limited to:



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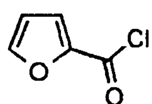

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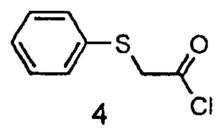

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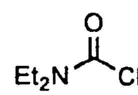

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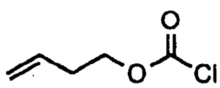

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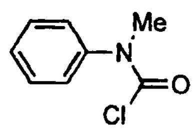

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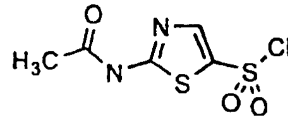

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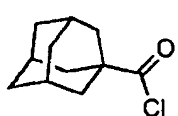

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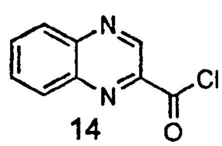

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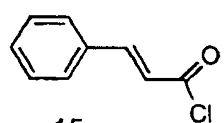

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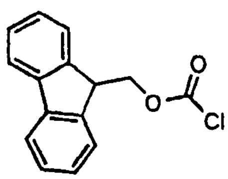

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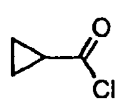

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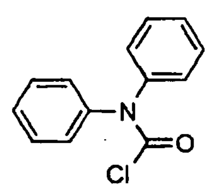

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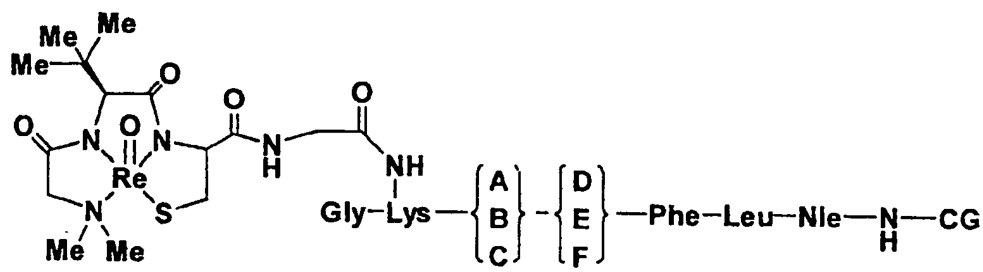
<u>Example 18:</u> **Synthesis of a library of 324 potential chemotactic peptides using a combination of parallel synthesis and split and mix technologies**

Libraries are based on the following peptide sequence:



RP#	Mixture A,B,C	Mixture D,E,F	Capping Group
552-6	Phe, Asp, Leu	Trp, Ser, Tyr	N,N-diethycarbamyl
552-10	Phe, Asp, Leu	Trp, Ser, Tyr	N-phenyl,N-methylcarbamyl
552-13	Phe, Asp, Leu	Trp, Ser, Tyr	Adamantylcarbonyl
552-16	Phe, Asp, Leu	Trp, Ser, Tyr	Fluorenylmethylcarbonyl
552-17	Phe, Asp, Leu	Trp, Ser, Tyr	Cyclopropylcarbonyl
552-18	Phe, Asp, Leu	Trp, Ser, Tyr	N,N-diphenylcarbamyl
553-6	Glu, His, Lys	Trp, Ser, Tyr	N,N-diethycarbamyl
553-10	Glu, His, Lys	Trp, Ser, Tyr	N-phenyl,N-methylcarbamyl
553-13	Glu, His, Lys	Trp, Ser, Tyr	Adamantylcarbonyl
553-16	Glu, His, Lys	Trp, Ser, Tyr	Fluorenylmethylcarbonyl
553-17	Glu, His, Lys	Trp, Ser, Tyr	Cyclopropylcarbonyl
553-18	Glu, His, Lys	Trp, Ser, Tyr	N,N-diphenylcarbamyl
554-6	Asn, Arg, Val	Glu, His, Lys	N,N-diethycarbamyl
554-10	Asn, Arg, Val	Glu, His, Lys	N-phenyl,N-methylcarbamyl
554-13	Asn, Arg, Val	Glu, His, Lys	Adamantylcarbonyl
554-16	Asn, Arg, Val	Glu, His, Lys	Fluorenylmethylcarbonyl
554-17	Asn, Arg, Val	Glu, His, Lys	Cyclopropylcarbonyl
554-18	Asn, Arg, Val	Glu, His, Lys	N,N-diphenylcarbamyl

555-6	Phe, Asp, Leu	Asn, Arg, Val	N,N-diethycarbamyl
555-10	Phe, Asp, Leu	Asn, Arg, Val	N-phenyl,N-methylcarbamyl
555-13	Phe, Asp, Leu	Asn, Arg, Val	Adamantylcarbonyl
555-16	Phe, Asp, Leu	Asn, Arg, Val	Fluorenylmethylcarbonyl
555-17	Phe, Asp, Leu	Asn, Arg, Val	Cyclopropylcarbonyl
555-18	Phe, Asp, Leu	Asn, Arg, Val	N,N-diphenylcarbamyl
556-6	Trp, Ser, Tyr	Asn, Arg, Val	N,N-diethycarbamyl
556-10	Trp, Ser, Tyr	Asn, Arg, Val	N-phenyl,N-methylcarbamyl
556-13	Trp, Ser, Tyr	Asn, Arg, Val	Adamantylcarbonyl
556-16	Trp, Ser, Tyr	Asn, Arg, Val	Fluorenylmethylcarbonyl
556-17	Trp, Ser, Tyr	Asn, Arg, Val	Cyclopropylcarbonyl
556-18	Trp, Ser, Tyr	Asn, Arg, Val	N,N-diphenylcarbamyl
557-6	Ile, Gln, Thr	Asn, Arg, Val	N,N-diethycarbamyl
557-10	Ile, Gln, Thr	Asn, Arg, Val	N-phenyl,N-methylcarbamyl
557-13	Ile, Gln, Thr	Asn, Arg, Val	Adamantylcarbonyl
557-16	Ile, Gln, Thr	Asn, Arg, Val	Fluorenylmethylcarbonyl
557-17	Ile, Gln, Thr	Asn, Arg, Val	Cyclopropylcarbonyl
557-18	Ile, Gln, Thr	Asn, Arg, Val	N,N-diphenylcarbamyl

The various peptide sequences (that is the sequence Gly-Lys(DDE)-(mixture A,B,C)-(mixture D,E,F)-Phe-Leu-Nle-NH_2 and numbered RP552 through RP557) containing varying side chain protecting groups in these examples were synthesised via a solid phase synthesis method on an automated synthesizer using FastMoc chemistry on 1.0 mmol scale. Prior to the addition of each amino acid residue (or mixture of acids as described above) to the N-terminus of the peptide chain the FMOC group was removed with 20% piperidine in NMP. Each FMOC amino acid

was activated with 0.5M HOBT/HBTU/DMF in the presence of 2.0M DIEA/NMP. The C-terminus was attached to the solid phase *via* the sasrin linker. After completion of the synthesis the resin was washed with NMP followed by dichloromethane and dried under vacuum for up to 24 hours. Where mixture of amino acids were employed the three amino acids were added as equimolar mixtures of suitably side chain protected FMOC acid residues in a single coupling step and otherwise treated as a single amino acid residue.

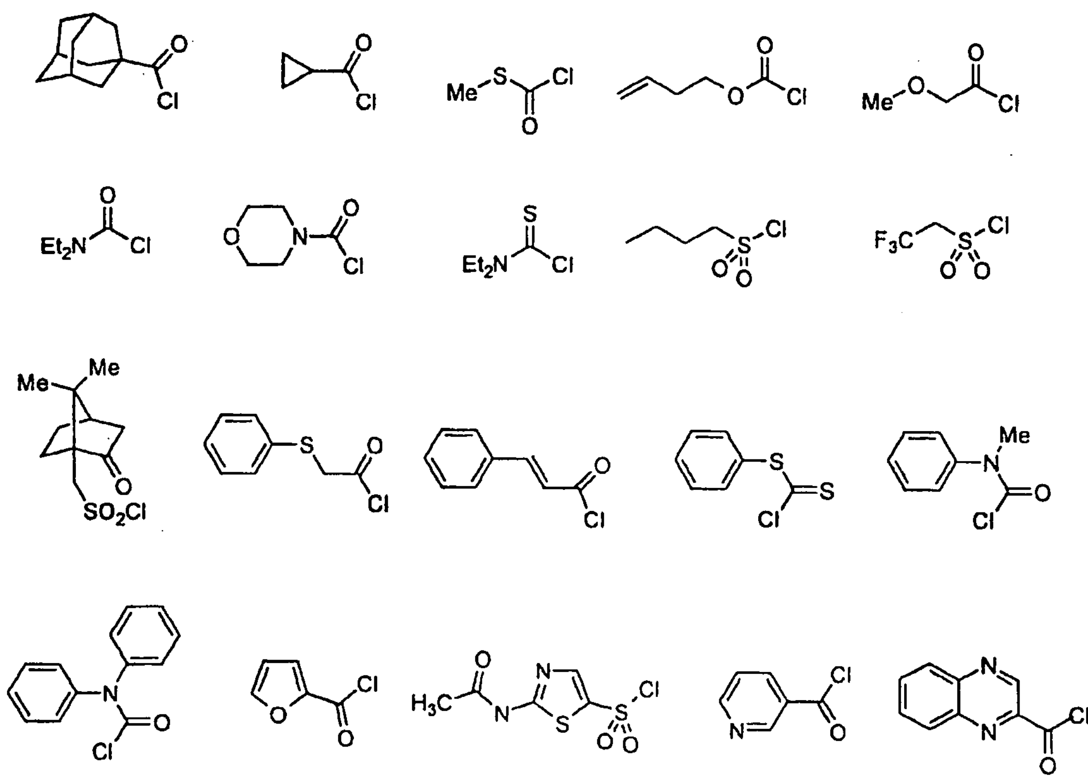
Example 19: **Capping the terminal amino group of peptide mixtures (RP552-557)**

The N-terminal amino group of each mixture of 9 compounds is deprotected of its Fmoc group under standard fmoc deprotection conditions. The amine is then capped with a suitably reactive reagent (see list). Each microkan is placed in a solution of NMP (500uL) for the resin to swell. To this solution is added DIEA (25uL, 2M) and one of the capping agents (0.5mmol). The reaction is shaken for 2 to 18 hours at room temperature. Completion of the capping was confirmed by treatment of a small portion of the resin with 3% ninhydrin-EtOH. Lack of blue/purple colour indicated a complete reaction. The reaction is filtered and the resin is washed with NMP (3x5mL) then dichloromethane (3x5mL) and the resin is dried in vacuo.

Each of the peptides was liberated from the support in 95% TFA: 5% water (1mL) after 4 hours shaking at room temperature, followed by filtration. The products were concentrated *in vacuo*. The residue was redissolved in trifluoroacetic acid (150uL) and dripped into t-butyl-methyl ether (5mL) to precipitate. Each was centrifuged to a pellet, the solvent decanted and the pellets dried in vacuo. The products were dissolved in water and acetontrile (~5mL) and lyophilized to pale beige powders. The products were then purified by reverse phase HPLC (C18 silica Gel using a gradient system of 0-to 100% acetonitrile in water buffered with 0.1% trifluoroacetic acid), the products having the retention times and mass spectra as described below in the table.

A list of such amine capping agents might include but is not limited to:

List of Capping Groups for Chemotactic Peptide



Example 20: **Routes for the introduction of the rhenium complexes to each mixture (RP552-557)**

Two routes to the introduction of a rhenium complex to the mixtures can be envisaged.

Route A:

This route requires that the lysine side chain first be deprotected and one of the chelators (RP414 or RP455) be attached to the sequence as a single residue or stepwise. Each of these can be accomplished by standard synthesizer chemistry. The cyst ine residue must be sulphur protected with a labile group which is lost

during rhenium coordination such as Mott as described in example 7 of the original patent filing.

Each mixture containing the rhenium chelator is placed in a solution of NMP (3mL) and the resin is allowed to swell. To each is added $ReOCl_3(PPh_3)_2$ (0.5mmol) and triethylamine (1mL). The reaction is heated at 40-50°C for 8 hours. The reaction is filtered and the resin is washed with NMP (3x5mL) then dichloromethane (3x5mL) and dried in vacuo.

Route B:

This route requires that the lysine side chain be deprotected on the resin and the entire rhenium complex (RP414 or RP455) be attached as a single residue Each microkan was placed in NMP (1mL) to swell the resin. To the solution was added ReO-RP414 (20mg, 0.037mmol) or ReO-RP455 (0.037mmol), then tetrafluorophenol (10mg, 0.06mmol), and EDC (20mg, 0.1mmol). The reaction is shaken for 24 hr in a 45°C bath. The reaction is filtered and the resin is washed with NMP (3x5mL) then dichloromethane (3x5mL) and dried in vacuo. Alternately, several mikrocans can be reacted at the same time in a larger reaction volume in the same stoichiometric ratios.

Example 21: **Synthesis of ReO-Dimethylglycine-t-butyl-glycine-S Acetamidomethyl-Cysteine-Glycine(ReO-RP455)**

The title product was synthesized by the literature methods of E. Wong et al. Inorganic Chemistry 36: 5799-5808 (1997).

Example 22: **Synthesis of ReO-Dimethylglycine-t-butyl-glycine-S-Acetamidomethyl-Cysteine-Glycine(ReO-RP455) tetrafluorophenyl ester**

To ReO-RP455 (60mg) in 1:1 acetonitrile:water (1mL) was added tetrafluoroph nol (100mg). The solution was diluted with acetonitrile (2mL). The pH

was measured at 2. To the solution was added 1(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride. The reaction was swirled to dissolve and the pH measured at 5. The reaction was allowed sit at room temperature for 15 minutes followed by concentrating to a dark oil *in vacuo*. The product was purified on a Supelco supelclean LC-18 column. The column was first washed with a 5% acetonitrile: 95% water solution acidified to pH2 with 3N HCl. The product was eluted in a 50% acetonitrile: 50% water solution acidified to pH2 with 3N HCl. The appropriate pure fractions were identified by silica TLC (t-butanol:water:methanol, 10:3:2, rf: 0.85) followed by $KMnO_4$ staining. The correct fractions were pooled and concentrated *in vacuo* to a red-brown glass (58mg, % yield).

Example 23: **Synthesis of ReORP455 libraries RP552 to RP557 (N-terminus amino group capped with capping groups 6, 10, 13, 16, 17, 18) on sasrin resin**

Each of the N-terminus capped libraries on sasrin resin (20 mg) was placed in a Biorad disposable column. The Dde epsilon amino group protection on C-terminus Lysine was first removed with three washes of 2% hydrazine in N-methylpyrrolidone (3x1mL). The resin was thoroughly washed with N-methylpyrrolidone then dichloromethane, and dried *in vacuo*. To each vessel was added ReO-Dimethylglycine-t-butyl-glycine-S-Acetamidomethyl-Cysteine-Glycine(ReO-RP455) tetrafluorophenyl ester (10mg) in ethyl acetate (1mL). The reactions were capped and shaken 20 hours at room temperature, followed by filtration, washing with copious ethyl acetate, N-methylpyrrolidone, dichloromethane. The red-brown resins were dried *in vacuo*.

Example 24: **Cleavage of ReO-RP455-RP552 to RP557 with N-terminus amino capping groups attached**

Each of the ReO libraries were liberated from the supports in 95% TFA: 5% water (1mL) after 4 hours shaking at room temperature, followed by filtration. The products were concentrated *in vacuo*. The residue was redissolved in trifluoroacetic

acid (150uL) and dripped into t-butyl-methyl ether (5mL) to precipitate. Each was centrifuged to a pellet, the solvent decanted and the pellets dried in vacuo. The products were dissolved in water and acetontrile (~5mL) and lyophilized to pale pink powders.

Example 25: **Deconvolution of Peptide Mixture ReORP552 with N-terminus capping goups attached.**

Following assay of the above mixtures of compounds using the method detailed below the following series of peptides was prepared as single molecules using the following method. The various peptide sequences containing varying side chain protecting groups in these examples were synthesized via a solid phase synthesis method on an automated synthesizer using FastMoc chemistry on scales varying from 0.1 to 1.0mmol. Prior to the addition of each amino acid residue to the N-terminus of the peptide chain the FMOC group was removed with 20% piperidine in NMP. Each FMOC amino acid was activated with 0.5M HOBT/HBTU/DMF in the presence of 2.0M DIEA/NMP. The C-terminus was attached to the solid phase *via* the sasrin linker. After completion of the synthesis the resin was washed with NMP followed by dichloromethane and dried under vacuum for up to 24 hours.

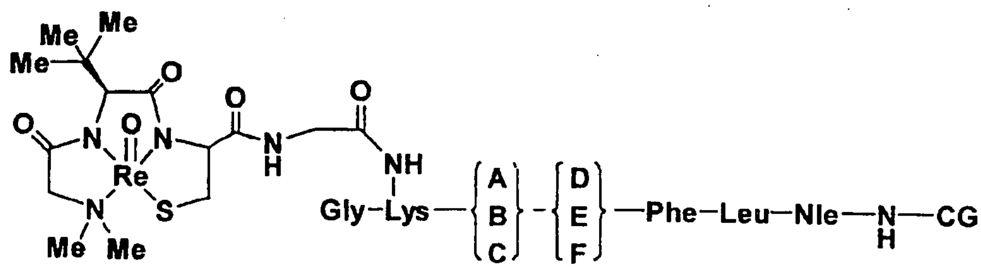
The following peptides were prepared and used still attached to sasrin resin:

Resin-gly-lys(DDE)-glu-trp-phe-leu-Nle
Resin-gly-lys(DDE)-glu-ser-phe-leu-Nle
Resin-gly-lys(DDE)-glu-tyr-phe-leu-Nle
Resin-gly-lys(DDE)-his-trp-phe-leu-Nle
Resin-gly-lys(DDE)-his-ser-phe-leu-Nle
Resin-gly-lys(DDE)-his-tyr-phe-leu-Nle
Resin-gly-lys(DDE)-lys-trp-phe-leu-Nle
Resin-gly-lys(DDE)-lys-ser-phe-leu-Nle
Resin-gly-lys(DDE)-lys-tyr-phe-leu-Nle

Each of the following resins containing the peptides was then capped with a cyclopropylcarbonyl group as follows: Each resin is placed in a solution of NMP (500uL) for the resin to swell. To this solution is added DIEA (25uL, 2M) and cyclopropane carbonyl chloride (0.5mmol). The reaction is shaken for 18 hours at room temperature. The reaction is filtered and the resin is washed with NMP (3x5mL) then dichloromethane (3x5mL) and the resin is dried in vacuo. Completion of the reaction is ensured by the use of a ninhydrin test to indicate complete reaction of amino groups.

Having attached the capping group to the amino terminus of the peptide attached to the resin the rhenium complex was introduced as follows: The Dde epsilon amino group protection on C-terminus Lysine was first removed with three, five minute washes of 2% hydrazine in N-methylpyrrolidone (3x1mL). The resin was then thoroughly washed with N-methylpyrrolidone then dichloromethane, and dried *in vacuo*. To each vessel was added ReO-Dimethylglycine-t-butyl-glycine-S-Acetamidomethyl-Cysteine-Glycine(ReO-RP455) tetrafluorophenyl ester (10mg) in ethyl acetate (1mL). The reactions were capped and shaken 20 hours at room temperature, followed by filtration, washing with copious ethyl acetate, N-methylpyrrolidone, dichloromethane. The red-brown resins were dried *in vacuo*. Liberation of the rhenium complex of the peptide from the resin was carried out as follows: Each of the ReO complexes were liberated from the supports in 95% TFA: 5% water (1mL) after 4 hours shaking at room temperature, followed by filtration. The products were concentrated *in vacuo*. The residue was redissolved in trifluoroacetic acid (150uL) and dripped into t-butyl-methyl ether (5mL) to precipitate. Each was centrifuged to a pellet, the solvent decanted and the pellets dried in vacuo. The products were dissolved in water and acetontrile (~5mL) and lyophilized to pale pink powders. The single compounds were then purified by reverse phase HPLC using vydac C18 protein and peptide column and a graded eluent system using water and acetonitrile buffered with 0.1% trifluoroacetic acid, the gradient increasing from 0% acetonitrile to 55% acetonitrile in water over a period of 20

minutes. The fractions containing the peptide were then lyophilized and analyzed by mass spectroscopy.



Compounds prepared as their rhenium oxo complex

ReO-Gly-lys(Dimethylgly-t-Butylgly-cys-gly)-glu-trp-phe-leu-Nle-NHCOcyclopropyl
HPLC retention time:22min; ESMS (1518, M+H), expected 1518

ReO-Gly-lys(Dimethylgly-t-Butylgly-cys-gly)-glu-ser-phe-leu-Nle-NHCOcyclopropyl
RP553-17-0 HPLC retention time: 19.8min

ReO-Gly-lys(Dimethylgly-t-Butylgly-cys-gly)-glu-tyr-phe-leu-Nle-NHCOcyclopropyl
RP553-17-0 HPLC retention time: 20.1min

ReO-Gly-lys(Dimethylgly-t-Butylgly-cys-gly)-his-trp-phe-leu-Nle-NHCOcyclopropyl
RP553-17-0 HPLC retention time: 21.0min

ReO-Gly-lys(Dimethylgly-t-Butylgly-cys-gly)-his-ser-phe-leu-Nle-NHCOcyclopropyl
RP553-17-0 HPLC retention time: 19.2min

ReO-Gly-lys(Dimethylgly-t-Butylgly-cys-gly)-his-tyr-phe-leu-Nle-NHCOcyclopropyl
RP553-17-0 HPLC retention time: 19.3min

ReO-Gly-lys(Dimethylgly-t-Butylgly-cys-gly)-lys-trp-phe-leu-Nle-NHCOcyclopropyl
RP553-17-0 HPLC retention time: 20.5 min

ReO-Gly-lys(Dimethylgly-t-Butylgly-cys-gly)-lys-ser-phe-leu-Nle-NHCOcyclopropyl
RP553-17-0 HPLC retention time: 18.8min

ReO-Gly-lys(Dimethylgly-t-Butylgly-cys-gly)-glu-tyr-phe-leu-Nle-NHCOcyclopropyl
RP553-17-0 HPLC retention time:

<u>Example 26:</u> **Small Molecule Re Complex Conjugate**

Using the methods described above the following peptide was prepared and used attached to the sasrin resin:

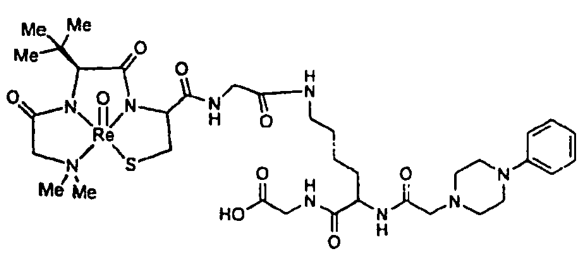
Resin-Gly-Lys(DDE)

Following the procedure described in Journal of the American Chemical Society 1992, 114, 10646 this peptide was treated with bromoacetic acid as follows:

To a slurry of the resin (0.5mmol/gram loading, 100mg) in dimethyformamide (0.5mL) was added bromoacetic acid (70mg, 0.5mmol) followed by dicyclohexylcarbodiimide (123mg, 0.6mmol). The resulting mixture was shaken at room temperature for 35 minutes and was then filtered. The resin was washed with dimethylformamide (3 x 2mL) followed by washing with dichloromethane (3 x 2mL) and was then dried in vacuo prior to further use. Ninhydrin test on this resin proved negative for free amino groups.

A portion of the above resin (20mg) was slurried in dimethysulfoxide (200 L) and to this slurry was added 1-phenylpiperazine (32mg, 30 L) and the whole mixture was agitated at room temperature for 4 hours. The mixture was filtered and the resin washed with N-methylpyrrolidinone (3 x 2mL) then dichloromethane (5 x 2mL) and

dried in vacuo. A small portion of this resin (7mg) was liberated from the resin using three successive fiv minute washes with 500 L of 2% hydrazine in N-methylpyrrolidinone (to remove the DDE protecting group) followed by 95% trifluoroacetic acid (1mL). Filtration of the TFA solution followed by removal of the volatiles under reduced pressure gave glycyl-lysyl-N-(4-phenylpiperazinylcarbonyl). The remainder of the resin was treated with 2% hydrazine as above (to remove the DDE group) and after washing and drying was then treated with a solution of ReO-Dimethylglycine-t-butyl-glycine-S-Acetamidomethyl-Cysteine-Glycine(ReO-RP455) tetrafluorophenyl ester (10mg) (prepared as described above) in ethylacetate (1mL) and the mixture shaken overnight. The resin was filtered and washed with N-methypyrrolidinone (3 x 2mL) followed by dichloromethane (3 x 2 mL) and then dried in vacuo. The rhenium complex was liberated from the resin using 95% trifluoroacetic acid (500 L) for 1.5h at room temperature. Filtration of the solution and removal of the trifluoroacetic acid under reduced pressure gave glycyl-lysine(-Re oxo Dimethylglycyl-t-Butylglycyl-cysteinyl-glycyl)-N-(4-phenylpiperazin-1-ylacetamide):ESMS 963 (M+H^{+}), expected 963.



Example 27: Preparation of libraries of small molecules

Using the method described above for the preparation of glycyl-lysine(-Re oxo Dimethylglycyl-t-Butylglycyl-cysteinyl-glycyl)-N-(4-phenylpiperazin-1-ylacetamide) libraries of small molecules have been prepared as follows. Equimolar mixtures of eight variously substituted piperazines were substituted for the phenylpiperazine in the above sequence to provide a final mixture of eight compounds. These mixtures are detailed below.

Mixture 1: 1-phenylpiperazine, 2-(1-piperazinyl)pyridine, 2-(1-piperazinyl)pyrimidine, 1-cyclohexylpiperazine, 1-(pyrrolidinocarbonylmethyl)piperazine, 1-(morpholinocarbonylmethyl)piperazine, 1-bis(4-fluorophenyl)methylpiperazine, 1-piperonylpiperazine

Mixture 2: 1-(2,3dimethylphenyl)piperazine, 1-(o-tolyl)piperazine, 1-(4-fluorophenyl)piperazine, 1-(4-nitrophenyl)piperazine, 1-(2-(2-hydroxyethoxy)ethyl)piperazine, 1-(4-chlorobenzhydryl)piperazine, 1-(4,4-bis(4-fluorophenyl)butyl)piperazine, 1-(diphenylmethyl)piperazine, 1-(2-hydroxyethyl)piperazine

Example 28: **Large Random Library of Re complex-peptide-conjugates**

Using the peptide synthesis method described previously, 6,859 peptides were synthesized with the following composition:

Sasrin-AA_1-AA_2-AA_3-β-Ala

Nineteen Sasrin resins with one amino acid attached (AA_1) were combined in the reaction vessel of the peptide synthesizer. The nineteen amino acids (AA2) included all of the natural amino acids except Cys. The synthesis was done on a 0.1 mmol scale. Therefore, 0.005 mmol of each resin-bound amino acid was added to the reaction vessel. The gram equivalents are listed in Table 1. Nineteen free amino acids were combined in equimolar amounts in each of two cartridges (AA_2 and AA_3). A 10-fold excess of each of the amino acids was used so that each cartridge contained 1mmol of amino acids. Therefore, there were 0.05 mmol of each amino acid in each of the cartridges.

The last amino acid to be added to the sequence was β-Ala. This acted as a linker for the Re oxo complex. The amount used was 1 mmol which is 0.311g.

Sasrin-AA_1	Mass (mg)	AA_2	Mass (mg)	AA_3	Mass (mg)
Gly	7.2	Gly	15.0	Gly	15.0
His(Trt)	12.5	His(Trt)	31.0	His(Trt)	31.0
Val	9.1	Val	17.0	Val	17.0
Tyr (tBu)	8.3	Tyr (tBu)	23.0	Tyr (tBu)	23.0
Trp (Boc)	8.3	Trp (Boc)	26.0	Trp (Boc)	26.0
Ala	7.3	Ala	16.0	Ala	16.0
Arg (Pmc)	11.0	Arg (Pmc)	33.0	Arg (Pmc)	33.0
Leu	8.3	Leu	18.0	Leu	18.0
Met	6.9	Met	18.0	Met	18.0
Phe	7.7	Phe	19.0	Phe	19.0
Pro	7.8	Pro	17.0	Pro	17.0
Ser (Trt)	8.3	Ser (Trt)	28.0	Ser (Trt)	28.0
Asn (Trt)	17.0	Asn (Trt)	30.0	Asn (Trt)	30.0
Thr (tBu)	11.0	Thr (tBu)	20.0	Thr (tBu)	20.0
Lys (Boc)	8.3	Lys (Boc)	23.0	Lys (Boc)	23.0
Ile	8.3	Ile	18.0	Ile	18.0
Glu (OtBu)	8.3	Glu (OtBu)	21.0	Glu (OtBu)	21.0
Gln (Trt)	12.5	Gln (Trt)	31.0	Gln (Trt)	31.0
Asp(OtBu)	8.8	Asp(OtBu)	21.0	Asp(OtBu)	21.0

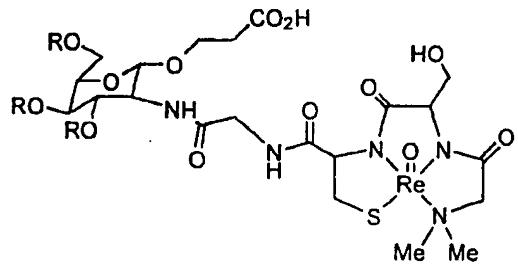
ReO-Dimethylglycine-serine-S-Acetamidomethyl-Cysteine-Glycine(ReO-RP414) tetrafluorophenyl ester (10mg) (prepared as described above for ReO-Dimethylglycine-serine-S-Acetamidomethyl-Cysteine-Glycine ReO RP455) ester was synthesized as described above and added as an ethyl acetate solution (in 1mL ethyl acetate) to the resin-bound peptide (10 mg). The reaction was allowed to mix overnight then was filtered and washed with ethyl acetate (3 times), water (3 times) and dichloromethane (3 times). The resin was dried on the aspirator and then cleaved with 95% trifluoroacetic acid in water (3 hours). The trifluoroacetic

acid/peptide solution was filtered into tert-butyl methyl ether (10 mL) and centrifuged. The ether was decanted. This washing process was repeated 3 times. After the final wash the ether was decanted leaving behind the peptide which had precipitated. The peptide was dissolved in water (1 mL), frozen with liquid nitrogen and lyophilized.

Example29: **Library of Rhenium compounds based on glycosides**

Compounds contained in this library rely on the structural constraints inherent in the carbohydrate structure to impart directionality into the compounds. Thus decoration of the carbohydrate skeleton with suitable functional groups, one of which consists of a rhenium chelating agent such as RP414 or RP455 as described above will allow the preparation of structurally diverse libraries of rhenium containing molecules. Hence treatment of glucose with a suitable alcohol (for example tertiary butanol or methanol) in acid catalyzed conditions (most preferably using Dowex 50W-X8 in benzene) following the procedure of Lin et. al. Journal of the American Chemical Society 1992, *114*, 10138 gives the glucose substituted at C-1. Treatment of this substituted glucose with a mixtures up to 20 diversity functionalities containing reactive leaving groups (for example iodide, triflate or tosylate) in an inert solvent (for example benzene or dichloromethane) results in a glucose having diversity attached at four points (i.e. C-2, C-3, C-4, C-5) around the periphery of the glucose ring. The rhenium containing moiety is then introduced by removal of the methoxy group at C-1 using water in acidic conditions. Application of the above glycosidation conditions with Re oxo dimethyglycyl-t-butylglycyl-cysteinyl-glycyl-4-hydroxypropyl produces a library of rhenium containing glycosides. These are then tested in biological assays and the most promising mixtures deconvoluted by the parallel synthesis of each of the single compounds in the mixture.

<u>Example 30:</u> **Construction of a solid phas library of 1,000 metallocarbohydrates for us as imaging agents**



Where R represents diversity groups as outlined in the procedure

Well established methods for organic synthesis are used to obtain a library of molecules having restrained conformation by virtue of a carbohydrate backbone. Hence glucosamine is protected as its Fmoc derivative by treatment with Fmoc-Cl and sodium bicarbonate in aqueous dioxane. The resulting protected amine is then treated with 3-hydroxypropionic acid and Dowex 50W-X8 in benzene according to the procedure of Lin et. al. Journal of the American Chemical Society **1992**, *114*, 10138 to give 2-fluorenylmethylcarbamoyl-1-(3-carboxypropyloxy)-glucosamine. This amino acid is then attached to sasrin resin using standard chemistry by coupling with HOBT/HBTU and N,N-diisopropylethylamine in NMP to provide a resin having the glucose attached via a propyl group at C-1. According to the procedure of M. J. Sofia, Journal of Organic Chemistry, **1998**, *63*, 2802 the resin so prepared is then treated with sodium hydride in tetrahydrofurn in the presence of the following to prepare a library of molecules substituted at C-3, C-4, and C-5 by ether linkages; 3-iodopropane, benzyliodide, 3-iodopropanol, 3-bromopropylamine, propargyl bromide, 2-bromomethylnaphthalene, 5-bromo-2-methyl-2-pentene, perfluoropropyl iodide, bromomethylcyclopropane, and 2-(2-bromoethyl)-2,5,5-trimethyl-1,3-dioxane. This solid phase library is then treated with 20% piperidine in NMP to remove the Fmoc group from the C-2 amino functionality and the resulting resin treated with a solution of ReO-N,N,-dimethylglycine-ser-cys-gly-tetrafluorophenyl ester to provide

the solid phas library. This library is then liberated from the solid support by treatment with 50% TFA in dichloromethane. Removal of the TFA and dichloromethane under vacuum followed by lyophilisation provides the metallocarbohydrate library.

Example 31: **Biological assays**

MATERIALS AND METHODS:

Animals and Reagents. Sprague-Dawley rats weighing 300-350 g were purchased from Charles River-Bausch & Lomb Laboratories (St.Constant, Quebec). Procedures followed standard Animal Care Committee protocols. The following drugs were used in this study: fMLP, N-t-BOC-methionyl-leucyl-phenylalanine (N-t-BOC-MLP), cytochalasin B, oyster shell glycogen, polyethylenimine, o-phenylenediamine (OPD), H_20_2 and H_2SO_4 (Sigma Chemical Corp., St. Louis, MO) and ^{3}H-fMLP (New England Nuclear, Boston, MA). Peptide fMLP derivatives, N-formyl-norleucyl—tyrosyl-lysine (For-Nle-LP-Nle-YK) and iso-boc-MLFK were synthesized in-house by Resolution Pharmaceuticals Inc. (Mississauga, ON).

Neutrophil isolation. Animals were sacrificed via administration of CO_2 4 hours after injection of 10 mL of 0.5% oyster glycogen. Leukocytes were harvested by performing peritoneal lavage using 30 mL of Hanks' buffered salt solution (HBSS-) containing 10 mM ethylene-diaminetetra-acetic acid (EDTA) disodium salt. The volume of fluid recovered from each rat was approximately 20-25 mL and bloody lavages were discarded.

Neutrophils isolated by peritoneal lavage were washed twice in HBSS- (without calcium chloride, magnesium chloride and magnesium sulfate). A cold H_20 RBC lysis was then performed with the addition of 9 mL of sterile ice-cold H_20 and 1 mL of phosphat buffered saline (PBS) solution containing 0.1 M phosphate buffer, 0.027 M KCl and 1.37 M NaCl. White blood cell differential stain was used to

identify neutrophils and viability of cells was confirmed on the basis of trypan blue exclusion.

Neutrophil fMLP receptor binding assays. fMLP saturation binding experiments used to determine K_D values were carried out with 2.5 X 10^5 PMNs per sample suspended in a final volume of 150 uL of fMLP, ^{3}H-fMLP and/or HBSS+. Samples were done in quadruplicate and non-specific binding was assessed in the presence of 10 *u*M fMLP and ^{3}H-fMLP in the range of 1 nM to 150 nM. Total binding was evaluated following the addition of ^{3}H-fMLP in the concentration range of 1 nM to 150 nM.

Competition assays were conducted with 6 nM ^{3}H-fMLP in addition to the nonradioactive competing ligand added at 10 *u*M and 1.0 *u*M. Total binding in the competition assays was assessed in the presence of 1.0 x 10^6 PMNs per sample while non-specific binding was determined in the absence of cells.

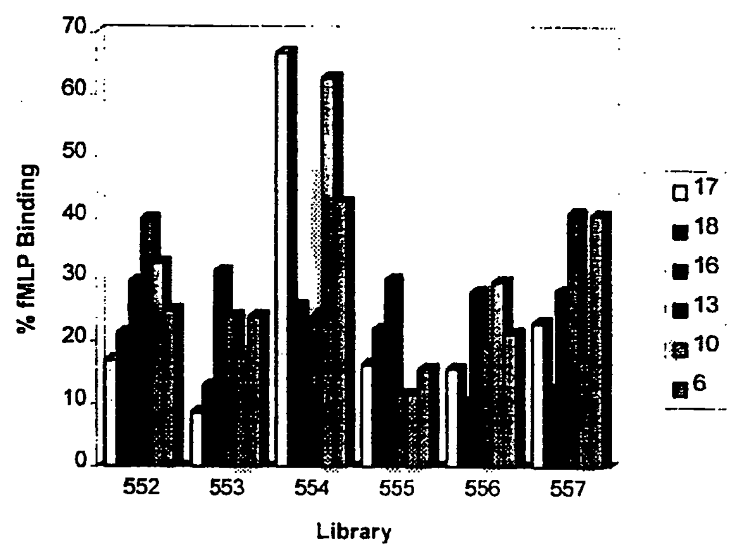
After a 1 hour incubation period on ice, the samples were vacuum aspirated onto 1.0 *u*m Skatron filter mats which had been pre-treated with polyethylenimine for approximately 24 hours. Sample wells received two consecutive 12 second washes with 0.9 % saline solution and collected filters were counted for 2 minutes in 5 mL of liquid scintillation fluid.

Measurement of Myeloperoxidase release. 0.5 x 10^6 PMNs per sample were incubated in 96-well Millipore Multiscreen 0.65 *u*m filter plates. In a final volume of 150 *u*L, 50 *u*L of the respective fMLP analogues (10 *u*M to 1pM) and/or fMLP (10 *u*M to 1.0 *u*M) were incubated with isolated PMNs pretreated with cytochalasin B (5 *u*g/mL) for 10 minutes. Following a 30 minute incubation period at room temperature, the supernatant was collected into a standard polypropylene plate with the Millipore vacuum apparatus. Supernatant samples were subsequently incubated with 50 uL o-phenylenediamine (OPD) containing H_2O_2 and HBSS+ for 2 minutes

followed by the addition of 2.5M H_2SO_4. $O.D._{490}$ values were obtained with the Th_rmomax Microplate Reader.

Graph Showing % fMLP remaining after challenge with mixtures of compounds prepared in Examples 18-24

Competition Binding of Libraries with fMLP



According to the above assays, library RP553-capped-17 was selected for further deconvolution.

Although the invention has been described with preferred embodiments, it is to be understood that modifications may be resorted to as will be apparent to those skilled in the art. Such modifications and variations are to be considered within the purview and scope of the present invention.

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WE CLAIM:

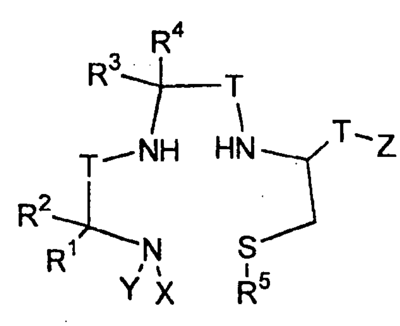
1. A library comprising one or more sets of compounds, each set comprising a mixture of compounds of formula (1):

$A\text{-}(B)_n\text{-}C$ (1)

wherein: A is a chelator complexed to a metal or a metal radionuclide or a chelator moiety capable of complexing a metal;
B is a spacer group;
n is selected from the integers 0 and 1; and
C comprises one of a plurality of potential targeting molecules.

2. The library of claim 2, wherein A is a chelator moiety capable of binding a metal selected from the group comprising polyamino polycarboxylates, polyamino polyphenolates, polyazamacrocycles with or without pendent coordination groups, tetradentate N_xS_{4-x} ligands, polyamino polysulfides, polyamino polyphosphates, polyamino polyheterocyclics, and derivatives or combinations thereof.

3. A library according to claim 1, wherein A is a metal chelator of the general formula:



wherein,

X is a lin ar or branched, saturated or unsaturated C_{1-6} alkyl chain that is optionally interrupted by one or two heteroatoms selected from N, O, and S; and is optionally substituted by at least one group selected from hydroxyl, amino, carboxyl, C_{1-6} alkyl, aryl and C(O)Z;

Y is H or a substituent defined by X;

Z is the position of attachment for the targeting portion of the library;

R^1 through R^4 are selected independently from H; carboxyl; C_{1-4} alkyl; C_{1-4} alkyl substituted with a group selected from hydroxyl, amino, sulfhydryl, halogen, carboxyl, C_{1-4} alkoxycarbonyl and aminocarbonyl; an alpha carbon side chain of a D- or L- amino acid other than proline; and C(O)Z;

R^5 is selected from H and a sulphur protecting group; and

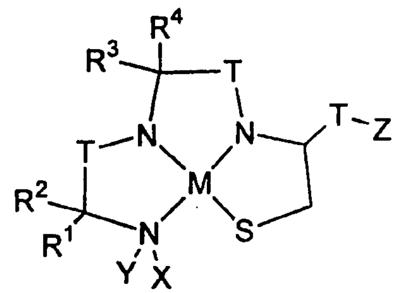
T is carbonyl or CH_2.

4. A library according to claim 3, wherein A is a metal chelator selected from the group comprising N,N-dimethylglycine-ser-cys-gly or N,N-dimethylglycine-tertbutylglycine-cys-gly.

5. A library according to claim 3 wherein A is a chelator complexed to a metal or metal radionuclide.

6. A library according to claim 5, wherein A is selected from the group comprising polyamino polycarboxylates, polyamino polyphenolates, polyazamacrocycles with or without pendent coordination groups, tetradentate N_xS_{4-x} ligands, polyamino polysulfides, polyamino polyphosphates, polyamino polyheterocyclics and derivatives or combinations thereof.

7. A library according to claim 5 wherein A is of th g neral formula:



X is a linear or branched, saturated or unsaturated C_{1-6} alkyl chain that is optionally interrupted by one or two heteroatoms selected from N, O, and S; and is optionally substituted by at least one group selected from hydroxyl, amino, carboxyl, C_{1-6} alkyl, aryl and C(O)Z;

Y is H or a substituent defined by X;

Z is the position of attachment for the targeting portion of the library;

R^1 through R^4 are selected independently from H; carboxyl; C_{1-4} alkyl; C_{1-4} alkyl substituted with a group selected from hydroxyl, amino, sulfhydryl, halogen, carboxyl, C_{1-4} alkoxycarbonyl and aminocarbonyl; an alpha carbon side chain of a D- or L- amino acid other than proline; and C(O)Z;

T is carbonyl or CH_2; and

M is metal.

8. A library according to claim 5 wherein A is selected from the group comprising: N, N-dimethylglycine-ser-cys-gly or N, N-dimethylglycine-tertbutylglycine-cys-gly.

9. A library according to claim 5 wherein the metal is selected from the group comprising: Sc, Ti, V, Cr, Mn, Fe, Co, Ni, Cu, Zn, Ga, Sr, Y, Zr, Nb, Mo, Tc, Ru, Rh, Pd, Ag, Cd, In, Hf, Ta, W, Re, Os, Ir, Pt, Au, Hg, Tl, Pb, Bi, La, Ce, Pr, Nd, Pm, Sm, Eu, Gd, Tb, Dy, Ho, Er, Tm, Yb, Lu.

10. A library according to claim 5 wherein wher in the metal radionuclide is selected from the group comprising: ^{99m}Tc, ^{99}Tc, ^{64}Cu, ^{67}Cu, ^{97}Ru, ^{109}Pd, ^{186}Re, ^{188}Re, ^{111}In, ^{113m}In, ^{153}Gd, ^{90}Y, ^{153}Sm, ^{166}Ho, ^{198}Au, ^{199}Au, ^{90}Sr, ^{89}Sr, ^{105}Rh, ^{201}Tl, ^{51}Cr, ^{67}Ga, ^{57}Co, ^{60}Co.

11. A library according to claim 5 wherein wherein the metal radionuclide is selected from the group comprising: ^{99m}Tc, ^{186}Re, ^{188}Re, ^{153}Sm, ^{111}In, ^{90}Y, ^{166}Ho.

12. A library according to claim 1 wherein C comprises one of a plurality of potential targeting molecules showing either agonist or antagonist activity selected from the group comprising: proteins, peptides, nucleotides, oligonucleotides, saccharides, oligosaccharides, steroids, cyclic peptides, peptidomimetics, enzyme substrates and inhibitors and small organic molecules (acyclic, cyclic and heterocyclic).

13. A library according to claim 1 wherein C comprises one of a plurality of potential targeting molecules selected from the group comprising: peptides, saccharides, cyclic peptides, peptidomimetics and small organic molecules.

14. A library comprising one or more sets of compounds, each set comprising a mixture of compounds of formula (11):

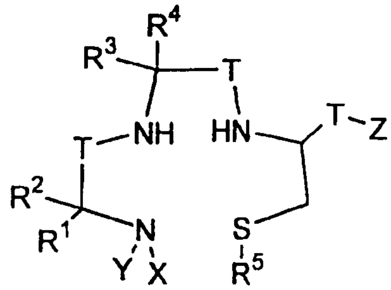
$$(W)_m\text{-}X\text{-}(Y)_n\text{-}Z \qquad (11)$$

Wherein: **W** is selected from a group comprising:

a) a metal binding moiety;

b) a chelator moiety capable of binding a metal selected from polyamino polycarboxylates, polyamino polyphenolates, polyazamacrocycles with or without pendent coordination groups, tetradentate N_xS_{4-x} ligands, polyamino polysulfides, polyamino polyphosphates, polyamino polyheterocyclics and derivatives or combinations of the above;

c) a metal ch lator of the general formula;



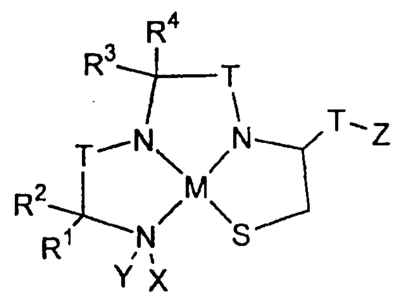
X is a linear or branched, saturated or unsaturated C_{1-6} alkyl chain that is optionally interrupted by one or two heteroatoms selected from N, O,and S; and is optionally substituted by at least one group selected from hydroxyl, amino, carboxyl, C_{1-6} alkyl, aryl and C(O)Z;
Y is H or a substituent defined by X;
Z is the position of attachment for the targeting portion of the library;
R^1 through R^4 are selected independently from H; carboxyl; C_{1-4} alkyl; C_{1-4} alkyl substituted with a group selected from hydroxyl, amino, sulfhydryl, halogen, carboxyl, C_{1-4} alkoxycarbonyl and aminocarbonyl; an alpha carbon side chain of a D- or L- amino acid other than proline; and C(O)Z;
R^5 is selected from H and a sulphur protecting group; and
T is carbonyl or CH_2;

d) a metal chelator selected from N,N-dimethyglycine-ser-cys-gly or N,N-dimethylglycine-tertbutylglycine-cys-gly; and
e) a chelator complexed to a metal or metal radionuclide;

X is a multiple chelator binding moiety capable of coupling to at least one metal binding moiety;
Y is a spacer group is selected from the integers 0 and 1;
Z comprises a mixture of potential targeting moieties;
m is greater than or equal to 1; and

n is selected from the integers 0 and 1.

15. A library according to claim 14, wherein W is a chelator complexed to a metal or metal radionuclide of the general formula:



wherein,

X is a linear or branched, saturated or unsaturated C_{1-6} alkyl chain that is optionally interrupted by one or two heteroatoms selected from N, O,and S; and is optionally substituted by at least one group selected from hydroxyl, amino, carboxyl, C_{1-6} alkyl, aryl and C(O)Z;

Y is H or a substituent defined by X;

Z is the position of attachment for the targeting portion of the library;

R^1 through R^4 are selected independently from H; carboxyl; C_{1-4} alkyl; C_{1-4} alkyl substituted with a group selected from hydroxyl, amino, sulfhydryl, halogen, carboxyl, C_{1-4} alkoxycarbonyl and aminocarbonyl; an alpha carbon side chain of a D- or L- amino acid other than proline; and C(O)Z;

T is carbonyl or CH_2; and

M is a metal.

16. A library according to claim 14, wherein the metal is selected from the group comprising: Mn, Fe and Gd.

17. A library according to 14, wherein Z is selected from the group comprising proteins, peptides, nucleotides, oligonucleotides, saccharides, oligosaccharides, steroids, cyclic peptides, peptidomimetics and small organic molecules showing either agonist or antagonist activity.

18. A method for the synthesis of a library comprising one or more sets of compounds, each set comprising a mixture of compounds of formula 1:

$A\text{-}(B)_n\text{-}C$ (1)

wherein: A is a chelator moiety capable of complexing a metal;
B is a spacer group;
n is selected from the integers 0 and 1; and
C comprises one of a plurality of potential targeting molecules.

comprising of the steps of:

(1) Preparing a mixture of potential targeting molecules using combinatorial synthesis;

(11) Attaching to the mixture a metal chelating moiety capable of complexing a metal; and

(111) Complexing the mixture with a solution of the metal in a suitable solvent.

19. A method for the synthesis of a library comprising one or more sets of compounds, each set comprising a mixture of compounds of formula (1):

$A\text{-}(B)_n\text{-}C$ (1)

wherein: A is a chelator moiety capable of complexing a metal;
B is a spacer group;
n is selected from the integers 0 and 1; and
C comprises one of a plurality of potential targeting molecules.

comprising the steps of:

(1) Preparing a mixture of potential targeting molecules using combinatorial synthesis; and

(11) Attaching to the mixture a preformed metal complex as an activated reagent in a suitable solvent.

20. A method of obtaining a compound having a desired targeting property comprising the steps of:

(1) providing a mixture which comprises a set of candidate compounds of formula (1):

$A\text{-}(B)_n\text{-}C$ (1)

wherein;

A is a chelator complexed to a metal or metal nuclide

B is a spacer group;

n is selected from the integers 0 and 1; and

C is one of a plurality of potential targeting molecules; and

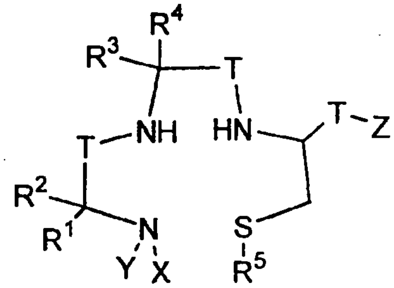
(11) selecting from amongst the set of candidate compounds a compound having the desired property by exposing the mixture of candidate compounds to a substance to which the compound having the desired targeting property will preferentially bind.

21. A method according to claim 20 wherein;

A is a chelator moiety capable of binding a metal selected from the group comprising polyamino polycarboxylates, polyamino polyphenolates, polyazamacrocycles with or without pendent coordination groups, tetradentate N_xS_{4-x} ligands, polyamino polysulfides, polyamino polyphosphates, polyamino polyheterocyclics and derivatives or combinations of the above.

22. A method according to claim 20 wherein;

A is a metal chelator of the general formula;



wherein,

X is a linear or branched, saturated or unsaturated C_{1-6} alkyl chain that is optionally interrupted by one or two heteroatoms selected from N, O,and S; and is optionally substituted by at least one group selected from hydroxyl, amino, carboxyl, C_{1-6} alkyl, aryl and C(O)Z;

Y is H or a substituent defined by X;

Z is the position of attachment for the targeting portion of the library

R^1 through R^4 are selected independently from H; carboxyl; C_{1-4} alkyl; C_{1-4} alkyl substituted with a group selected from hydroxyl, amino, sulfhydryl, halogen, carboxyl, C_{1-4} alkoxycarbonyl and aminocarbonyl; an alpha carbon side chain of a D- or L- amino acid other than proline; and C(O)Z;

R^5 is selected from H and a sulphur protecting group; and

T is carbonyl or CH_2.

23. A method according to claim 20 wherein,

A is a metal chelator selected from the group comprising N,N-dimethyglycine-ser-cys-gly and N,N-dimethylglycine-tertbutylglycine-cys-gly.

24. A method of obtaining a labeled compound for the purposes of therapy, radiotherapy or diagnostic imaging, having a desired targeting property comprising the steps of;

(1) providing one or more sets of mixtures which comprises a mixture of candidate compounds of formula (1):

$A\text{-}(B)_n\text{-}C$ (1)

wherein;

A is a chelator complexed to a metal or metal nuclide;

B is a spacer group;

n is selected from the integers 0 and 1; and

C is one of a plurality of potential targeting molecules; and

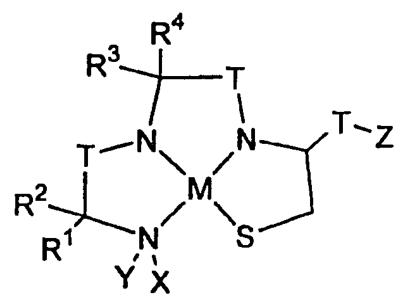
(11) selecting from among the set of candidate compounds a compound having the desired property by exposing the mixture of candidate compounds to a substance to which the compound having the desired targeting property will preferentially bind.

25. A method according to claim 24 wherein,

A is a metal complex of a chelator selected from the group comprising polyamino polycarboxylates, polyamino polyphenolates, polyazamacrocycles with or without pendent coordination groups, tetradentate N_xS_{4-x} ligands, polyamino polysulfides, polyamino polyphosphates, polyamino polyheterocyclics and derivatives or combinations thereof.

26. A method according to claim 24 wherein;

A is a metal complex of the general formula;



wherein,

X is a linear or branched, saturated or unsaturated C_{1-6} alkyl chain that is optionally interrupted by one or two heteroatoms selected from N, O, and S; and is optionally substituted by at least one group selected from hydroxyl, amino, carboxyl, C_{1-6} alkyl, aryl and C(O)Z;

Y is H or a substituent defined by X;

Z is the position of attachment for the targeting portion of the library;

R^1 through R^4 are selected independently from H; carboxyl; C_{1-4} alkyl; C_{1-4} alkyl substituted with a group selected from hydroxyl, amino, sulfhydryl, halogen, carboxyl, C_{1-4} alkoxycarbonyl and aminocarbonyl; an alpha carbon side chain of a D- or L- amino acid other than proline; and C(O)Z;

T is carbonyl or CH_2; and

M is a metal

27. A method according to claim 24 wherein;

A is a metal chelator selected from the group comprising N,N-dimethyglycine-ser-cys-gly and N,N-dimethylglycine-tertbutylglycine-cys-gly.

28. A method of obtaining a compound having a desired targeting property comprising the steps of;

(1) providing a mixture or set of mixtures which comprises a set of candidate compounds of formula (11):

$(W)_m$-X-$(Y)_n$-Z (11)

wherein: W is a metal binding moiety;

X is a multiple chelator binding moiety capable of coupling to at least one metal binding moiety;

Y is a spacer group is selected from the integers 0 and 1; and

Z comprises a mixture of potential targeting moieties;

m is greater than or equal to 1; and

n is selected from the integers 0 and 1; and

(11) selecting from among the set of compounds a compound having the desired targeting property by exposing the mixture of compounds to a substance to which the compound having a desired targeting property will preferentially bind.

29. A method of obtaining a molecule having a desired targeting property comprising the steps of:

(1) preparing a mixture or set of mixtures of candidate compounds of general formula (1):

A-$(B)_n$-C (1)

wherein;

A is a chelator complexed to a non-radioactive metal which is isostructural with an analogous complex of a radioactive metal;

B is a spacer group;

n is selected from the integers 0 and 1; and

C is one of a plurality of potential targeting molecules;

(11) selecting from among the set of candidates a compound having the desired targeting property by exposing the mixture of candidate compounds to a substance to which the compound will preferentially bind; and

(111) preparing the isostructural radioactive analogue of the selected candidate having the desired targeting property.

30. A method for the synthesis of a library comprising one or more sets of compounds comprising the steps of:

(I) Selecting a suitable targeting molecule for binding a biological target;

(II) Preparing a library of non-radioactive rhenium-targeting molecule conjugates;

(III) Dividing mixtures of the conjugates into separate wells;

(IV) Assaying the mixtures for binding affinity to the biological target;

(V) Deconvoluting the mixtures having a high a binding affinity for said biological target; and

(VI) Isolating a series of discrete compounds having a high a binding affinity for said biological target.

31. A method according to claim 30 further comprising the steps of:

(I) Substituting non-radioactive rhenium for radioactive technetium which isostructural to the non-radioactive rhenium; and

(II) D livering the technetium-targeting molecule conjugates for radiolabelling development in *in vivo* studies.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 98/00801

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K51/04 A61K51/08 A61K49/00 C07K1/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07B C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BURGER M T ET AL: "SYNTHETIC IONOPHORES. ENCODED COMBINATORIAL LIBRARIES OF CYCLEN-BASED RECEPTORS FOR CU2+ AND CO2+" JOURNAL OF ORGANIC CHEMISTRY, vol. 60, no. 23, 17 November 1995, page 7382/7383 XP002051768	1,2,12, 13,20,21
Y	see the whole document	1-13, 20-27

X	FRANCIS M B ET AL: "COMBINATORIAL APPROACH TO THE DISCOVERY OF NOVEL COORDINATION COMPLEXES" JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol. 118, no. 37, 18 September 1996, page 8983/8984 XP002051766	1,2,12, 13
Y	see the whole document	1-13, 20-27

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
29 January 1999	08/02/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Veronese, A

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INTERNATIONAL SEARCH REPORT

International Application No
PCT/CA 98/00801

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 96 03427 A (RESOLUTION PHARM INC ;POLLAK ALFRED (CA); GOODBODY ANNE (CA)) 8 February 1996 see claims 1-30; examples 1-4	1-13, 20-27
Y	WO 95 22996 A (RESOLUTION PHARM INC ;GOODBODY ANNE (CA); POLLAK ALFRED (CA)) 31 August 1995 see page 7, line 10 - page 9, line 5; claims; examples 1,2	1-13, 20-27
P,Y	DE 197 01 665 A (HANS KNOELL INST FUER NATURSTO ;UNIV SCHILLER JENA (DE)) 23 July 1998 see claims 1-4,6-12	1-13, 20-27
A	TERRETT N K ET AL: "COMBINATORIAL SYNTHESIS - THE DESIGN OF COMPOUND LIBRARIES AND THEIR APPLICATION TO DRUG DISCOVERY" TETRAHEDRON, vol. 51, no. 30, 24 July 1995, pages 8135-8173, XP002025141 see the whole document	1-30
A	WIENER E C ET AL: "DENDRIMER-BASED METAL CHELATES: A NEW CLASS OF MAGNETIC RESONANCE IMAGING CONTRAST AGENTS" MAGNETIC RESONANCE IN MEDICINE, vol. 31, no. 1, 1 January 1994, pages 1-8, XP000423671 see the whole document	14-17,28

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CA 98/ 00801

Box I Observations where certain claims were found unsearchable (Continuati n of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 31
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 31 is directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/CA 98/00801

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9603427 A	08-02-1996	US 5662885 A	02-09-1997
		AU 2301195 A	22-02-1996
		CA 2194551 A	08-02-1996
		CN 1158133 A	27-08-1997
		EP 0772628 A	14-05-1997
		HU 77137 A	02-03-1998
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WO 9522996 A	31-08-1995	US 5569745 A	29-10-1996
		AU 1803395 A	11-09-1995
		CA 2182670 A	31-08-1995
		EP 0746340 A	11-12-1996
		JP 9509419 T	22-09-1997
		US 5679642 A	21-10-1997
DE 19701665 A	23-07-1998	NONE	